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VIRAL PROTEINS

1. CLEAVAGE OF RAUSCHER LEUKAEMIA VIRUS (R-MULV) PR65GAG BY THE MOLONEY LEUKAEMIA VIRUS (M-MULV) PROTEOLYTIC ACTIVITY PRODUCES THE R-MULV-SPECIFIC BUT NOT THE M-MULV-SPECIFIC 40,000 DALTON INTERMEDIATE POLYPEPTIDE.

Yoshinaka Y, Luftig RB
Dept. Microbiology, Mie Univ. Sch. Medicine, 174 Edobashi-2-Chome, Tsu, Mie, Japan 514
J Gen Virol; 54(part1):33-38 1981

The discovery of a 1,500 dalton mol wt difference in Moloney leukemia virus (M-MuLV) and Rauscher leukemia virus (R-MuLV) intermediate products was used to monitor the results of in vitro cleavage of R-MuLV cpre precursor polyprotein Pr65gag by the proteolytic activity present in M-MuLV extracts. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that cleavage yields an MuLV Pr40gag polypeptide; at no point during the reaction with increasing amounts of the M-MuLV extract containing the proteolytic activity was any material found in a band position cor-

HE 20. 3173/2:

responding to the M-MuLV protein of 1,500 dalton higher mol wt, named Pr41.5gag. The possibility of a rapid cleavage of R-MuLV Pr65gag t o MuLV Pr41.5gag, then to its antigenic determinant p30, was ruled out by cleaving 35S-methionine-R-MuLV Pr65gag with the unlabeled M-MuLV extract. Only labeled MuLV Pr40gag and no labeled MuLV Pr41.5gag was found. A subspecies difference in size between M-MuLV Pr41.5gag and R-MuLV Pr40gag is apparent; if M-MuLV extracts contain only one Pr65gag protease, the fact that M-MuLV extract proteolytic activity cleaves R-MuLV Pr65gag to R-MuLV Pr40gag and not to M-MuLV Pr41.5gag suggests that the specificity for initial cleavage is built into the corresponding Pr65gag precursors and not into the corresponding proteolytic factors which supply the activity. (11 Refs)

2. TWO MURINE LEUKEMIA VIRUS PROTEIN KINASE ACTIVITIES AND THEIR POSSIBLE FUNCTIONS (MEETING ABSTRACT).

Yoshinaka Y, Luftig RB
Department of Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC, 29208
RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

Using an in vitro phosphorylation assay with detergent treated virus or core subparticles as substrate, we have found that: (1) Murine leukemia viruses, as avian leukemia viruses contain two protein kinase activities; one is preferentially acidic protein-accepting and the other prefers basic protein substrates. These can be separated by Sepharose 68 column chromatography. (2) The murine acidic kinase activity preferentially phosphorylates (80%) Pr65gag, in contrast to other virion proteins. In vitro phosphorylation of Pr65gag probably occurs at the p12 site since, when such Pr65gag is cleared in vitro, it produces as its initial product a band that has the Mr of Pr65gag and the antigenic determinants of p15 and p12. These results also indicate that the uncleaved Pr65gag packaged into virions is relatively unphosphorylated and is consistent with the contention of Naso et al that if Pr65gag is phosphorylated in vivo it is rapidly cleaved (3) The basic murine kinase activity preferentially phosphorylates the histone-like protein, MuLV p10; this appears to be correlated with release of the ribonucleoprotein from stable mature cores. Such a phosphorylation would most likely be related to uncoating of the virus particle after it has entered the cytoplasm of the infected mouse cell. (2 Refs)

3. IN VITRO PROCESSING OF GAZDAR MURINE SARCOMA VIRUS (GZ-MSV) ASSOCIATED PR65GAG BY THE PARTIALLY PURIFIED MOLONEY LEUKEMIA VIRUS (MOLV) PROTEOLYTIC FACTOR (MEETING ABSTRACT).

Yoshinaka Y, Luftig RB
Department of Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC, 29208
RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

Electron microscopy of Gz-MSV showed that almost 100% of the particles possessed an immature morphology. As expected from our earlier results sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by immuno-replica electrophoresis of Gz-MSV showed that Pr65gag (p15, p12, p30 and p10) was the predominant protein moiety of the virus. There was no gp70 present in these virus particles. In vitro processing of Pr65gag for about one hr, using a proteolytic factor that had been partially purified from MoLV by Sephadex G-75 column chromatography, showed an initial production of Pr55gag (p15, p12, pr30), p40gag (p30, p10) and Pr27gag (p15, p12) intermediates. Additional incubation led to complete breakdown of Pr65gag to p30, p10, p15 and p12. The processing of Pr55gag and Pr40gag appeared to occur much more rapidly than that of Pr27gag. It thus seems that sequentially different processing events are involved in production of the four internal gs antigens from Gz-MSV Pr65gag. (2 Refs)

4. PRIMARY STRUCTURE OF THE LOW MOLECULAR WEIGHT NUCLEIC ACID-BINDING PROTEINS OF MURINE LEUKEMIA VIRUSES.

Henderson LE, Copeland TD, Sowder RC, Smythers GW, Oroszlan S
Biological Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD, 21701
J Biol Chem; 256(16):8400-8403 1981

Murine leukemia viruses contain a low mol wt basic protein, designated p10, which binds to single-stranded nucleic acids. The complete amino acid sequence of p10 from the Rauscher strain of virus has been determined. The partial amino acid sequences of p10s from Moloney, Friend, AKR, Gross, radiation leukemia, and BALB/2 viral strains have also been determined using microsequencing techniques. Rauscher p10 is composed of 56 amino acid residues; the other p10s are similar in size but differ from Rauscher by a few conservative amino acid substitutions. The structure of Rauscher p10 was compared to the structure of a functionally homologous protein from Rous avian sarcoma virus. The comparison revealed regions of amino acid sequence homologies which indicate a phylogenetic relationship between the murine and avian viral strains. The analyses revealed a periodic placement of three Cys residues and a Gly-His sequence. A structure involving these residues is found once in the murine protein and twice in the avian protein. A similar structure is seen in the single stranded nucleic acid binding protein of bacteriophage T4. However, in the latter case, the order of amino acid residues is inverted. (Author abstract) (56 Refs)

5. EFFECT OF ATP ON THE FRIEND MURINE LEUKEMIA VIRUS-ASSOCIATED ENDONUCLEASE ACTIVITY AND THE ENDONUCLEASE ACTIVITY OF THE AVIAN MYELOBLASTOSIS VIRUS RNA-DIRECTED DNA POLYMERASE.

Nissen-Meyer J, Raae AJ, Nes IF
Dept. Biochemistry, Univ. Bergen, Arstadveien 19, N-5000 Bergen, Norway
J Biol Chem; 256(15):7985-7989 1981

The Mn++-dependent endonuclease activity associated with the avian myeloblastosi s virus RNA-directed DNA polymerase has been shown to be activated by ATP in the presence of Mg++. In the presence of Mn++ the endonucleolytic activity was stimulated about three-fold by the addition of ATP. The earlier identified Mr = 40,000 Friend murine leukemia virus (F-MuLV)-associated endonuclease which functions in the presence of both Mg++ and Mn++ has also been shown to be similarly stimulated by ATP. For both endonuclease activities stimulation was only observed at ATP concentrations above 0.5 mM, and it did not increase upon elevating the ATP concentration above 2.5 mM. ADP and dATP also stimulated both activities, although not to the same extent as ATP. GTP had no apparent effect and AMP seemed to inhibit both activities. The effect ATP analogs had on the F-MuLV associated endonuclease activity could suggest that the endonuclease reaction in the presence of ATP might involve the cleavage of

beta-gamma phosphate bonds in ATP. Neither adenyl-5'-yl imidodiphosphate nor (beta,gamma-methylene)adenosine 5'-triphosphate stimulated the activity, whereas significant stimulation was observed in the presence of (alpha,beta-methylene)adenosine 5'-triphosphate. Although no ATPase activity could be detected in the purified F-MuLV endonuclease preparation, the data do not exclude the possibility that ATP may be cleaved in amounts which are equivalent to the number of nicks introduced into DNA by the virus-associated endonuclease. In the presence of ATP and Mg++ the F-MuLV-associated endonuclease nicked both supercoiled and linear DNA duplexes extensively, although the former was nicked more readily than the latter. Single-stranded DNA functioned poorly as a substrate. The nicks introduced by the enzyme contained a 5'-phosphoryl terminus and a 3'-hydroxyl group. (Author abstract) (23 Refs)

6. ANALYSIS OF THE AVIAN RETROVIRUS REVERSE TRANSCRIPTASE USING MONOCLONAL ANTIBODIES AND MOLECULAR CLONING PROCEDURES (MEETING ABSTRACT).

Weis JH, Faras AJ

Department of Microbiology, University of Minnesota, Minneapolis, MN

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

The structure and function of the avian retrovirus enzyme reverse transcriptase is being examined using monoclonal antibodies and molecular cloning. Monoclonal antibodies have been generated against the reverse transcriptase from avian myeloblastosis virus (AMV). Four of these antibodies will specifically immunoprecipitate reverse transcriptase from AMV and the B-77 strain of Rous sarcoma virus and will inhibit the polymerase activity of the enzyme. Using immunoaffinity chromatographic analysis the enzyme can be purified in a single step to greater than 95% homogeneity. In addition these antibodies are currently being used to determine the enzymatic activity present in specific proteolytic fragmen ts of the enzyme as well as the structure of the forms of the enzyme in infected cells. DNA sequencing analysis has determined the genomic position of the carboxyl end of the gag protein p15 and Bal 31 digestion is being utilized to correctly juxtapose the amino terminal portion of the polymerase gene in relation to an Escherichia coli lac promoter as well as various eukaryotic promotors for cloning into procaryotic and eukaryotic cells, respectively. (no Refs)

7. ROUS SARCOMA VIRUS GLYCOPROTEINS CONTAIN HYBRID-TYPE OLIGOSACCHARIDES.

Hunt LA, Wright SE

Dept. Microbiology and Immunology, Univ. Louisville Sch. Medicine, Louisville, KY, 40292

J Virol; 39(2):646-650 1981

Examination of [3H]mannose-labeled glycopeptides from Prague C Rous sarcoma virus gp85 with gel filtration and sequential glycosidase digestions demonstrated the presence of hybrid-type asparaginyl-oligosaccharides. The major hybrid species had an oligomannosyl core (Man5GlcNAc2-ASN) characteristic of neutral structures, plus 'branch' sugars (NeuNAc-Gal-GlcNAc-) characteristic of complex, acidic structures. (Author abstract) (12 Refs)

8. GLYCOPEPTIDES OF MURINE LEUKEMIA VIRUSES. III. GLYCOSYLATION OF ENV PRECURSOR GLYCOPROTEINS.

Kemp MC, Famulari NG, Compans RW

Dept. Microbiology, Univ. Alabama Medical Center, Birmingham, AL, 35294

J Virol; 39(2):463-470 1981

We have compared the glycopeptides obtained after extensive pronase digestion of the env precursors (PrENV proteins) of ecotropic, xenotropic, and dual-tropic murine leukemia viruses. Two glycopeptide size classes, having mol wt of approx 2,200 and 1,500, were shown to be associated with the PrENV proteins of all murine leukemia viruses studied. Glycopeptides associated with the env precursors were totally susceptible to endo-beta-N-acetyglucosaminidase H. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of partial endo-beta-N-acetylglucosaminidase H digestion products of the env precursor of dual-tropic mink cell focus-forming virus (MCF 247) revealed the presence of seven bands, suggesting that six glycosylation sites were present on the precursor molecule. The MCF 247 PrENV protein had been previously shown to be accessible to lactoperoxidase-catalyzed radioiodination on the surface of infected cells. The cell surface PrENV molecules had the same electrophoretic mobility as pulse-labeled PrENV protein, and after endo-beta-N-acetylglucosaminidase H treatment a similar shift in electrophoretic mobility was observed for the cell surface PrENV protein and the pulse-labeled precursors, a finding which indicated that the PrENV protein located on the cell surface also possessed only mannose-rich oligosaccharides. These results indicated that the env precursor glycoproteins of dual-tropic viruses had the unusual property of migrating to the cell surface without undergoing the normal oligosaccharide processing and proteolytic cleavage events that had been observed for ecotropic and xenotropic murine leukemia virus glycoproteins. (Author abstract) (20 Refs)

9. PURIFIED LOW-MOLECULAR-WEIGHT PROTEIN KINASE FROM MURINE SARCOMA VIRUS PARTICLES CATALYZES TYROSINE PHOSPHORYLATION ENDOGENOUSLY BUT PHOSPHORYLATES CELLULAR PROTEINS AT SERINE.

Sen A

Dept. Cellular Biology, Scripps Clinic and Res. Foundation, La Jolla, CA, 92037

J Virol; 39(2):612-624 1981

The low-mol-wt (LMW) protein kinase associated with high-titer murine sarcoma virions has been extensively purified by ammonium sulfate fractionation, Bio-Gel P-100 gel filtration, DEAE-cellulose and carboxymethyl cellulose chromatography. The purified enzyme migrates as a 16K polypeptide in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The enzyme catalyzes phosphotransfer with ATP as a phosphate donor to various exogenously added proteins as acceptors; it requires Mg++ and is independent of cyclic AMP. The enzyme preparation catalyzes a low level of phosphorylation in the absence of any exogenously added substrate and forms phosphotyrosine. However, in the presence of acceptor protein molecules including total soluble cytoplasmic proteins of murine sarcoma virus-transformed mouse cells, the phosphorylated end products contain predominantly phosphoserine. The virion-associated enzyme also shows a preference for phosphorylating certain polypeptides in the soluble cytoplasmic extracts of murine sarcoma virus-transformed cells. (Author abstract) (24 Refs)

10. THE SPECIFIC INTERACTION OF THE ROUS SARCOMA VIRUS TRANSFORMING PROTEIN, PP60SRC, WITH TWO CELLULAR PROTEINS.

Brugge JS, Erikson E, Erikson RL

Dept. Microbiology, Sch. Medicine, State Univ. New York, Stony Brook, Long Island, NY, 11794

Cell; 25(2):363-372 1981

Sera from rabbits bearing tumors induced by Rous sarcoma virus (RSV) were previously found to contain antibody to the RSV transforming protein, pp60src. Two additional transformation-specific phosphoproteins from RSV-transformed avian cells are immunoprecipitated with these sera. These proteins, having mol wt of 90,000 (pp90) and 50,000 (pp50), are not precipitated from uninfected or transformation-defective virus-infected cells and are not related to any RSV structural proteins. Normal uninfected chicken cells contain a protein that shares identical partial proteolytic cleavage peptides with the pp90 protein immunoprecipitated from RSV-transformed cells. This pp90 protein is one of the major cytoplasmic proteins in uninfected cells. Antibody directed against pp90 also immunoprecipitates pp60src and pp50 from lysates of RSV-transformed chicken cells. (Author abstract) (33 Refs)

VIRAL RNA

11. LOCALIZATION OF THE SPLICE JUNCTIONS IN THE SUBGENOMIC MRNAS OF ROUS SARCOMA VIRUS (MEETING ABSTRACT).

Schwartz D, Tizard R, Gilbert W, Taylor J, Guntaka R

Harvard University, Cambridge, MA

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

Putative splice points in RSV subgenomic RNAs have been located by S1 nuclease mapping with end-labeled DNA restriction fragments derived from cloned avian sarcoma virus DNA. We find potential leader sequence splice points approx 280, 580, 930 and 1130 nucleotides from the 5'-end of the genomic RNA. A comparison of the nucleotide sequence around each of these points with donor exonintron junction sequences predicted by the consensus rules indicates that the sites 930 and 1130 nucleotides from the 5'-end are most likely to participate in the formation of a spliced subgenomic mRNA. Preliminary Sl mapping of the envelope message acceptor site indicates that the leader sequence is spliced to the message body either 20, 60, or 160 nucleotides in front of the sequence coding for the mature gp85. The sequence around the 160 nucleotide location conforms best with the consensus rules for acceptor junctions. If translation of the env gene is initiated at the methionine codon present in 35S RNA in front of the coding sequence for mature gp85, the polypeptide produced will include a 64 amino acid signal peptide. A splice at any of the above sites, however, would mean that the initiating methionine codon for the env gene would be located in the spliced leader sequence. Sl nuclease mapping of the src message acceptor site indicates that a potential splice, which meets the requirements of the consensus rules for acceptor junctions, is located approx 120 nucleotides in front of the putative beginning of the src gene. (no Refs)

12. IDENTIFICATION OF A PACKAGED CELLULAR MRNA IN VIRIONS OF ROUS SARCOMA VIRUS.

Adkins B, Hunter T

Tumor Virology lab., Salk Inst., San Diego, CA, 92138

J Virol; 39(2):471-480 1981

A novel messenger activity has been identified by in vitro translation of the 70S virion RNAs of a variety of avian leukosis and avian sarcoma viruses. When the 70S virion RNA complex was heat dissociated and the polyadenylated RNA was fractionated on neutral sucrose gradients, a polypeptide of 34,000 daltons (34K) was observed in the translation products of 18S polyadenylic acid-containing virion RNA. Aside from the p60src-related subgenomic messenger activities, this was the only prominent messenger activity that sedimented at less than 20S. It was determined that the 34K protein was not virally coded because (i) messenger activity for the 34K protein was not generated by mild alkaline hydrolysis of 35S genomic RNA, (ii) the 34K proteins synthesized in response to different virion RNAs had identical tryptic peptide maps, and (iii) the tryptic peptide map of the 34K protein coded for by virion RNA was identical to that of a major in vitro translation product of 34,000 daltons made from 18S uninfected chick cell polyadenylated RNA. The 18S RNA was shown to be contained within virion particles, rather than part of a cellular structure copurifying with virus preparations, by demonstrating the presence of 34K messenger activity in virion cores made from detergent-disrupted virus. This cellular mRNA, however, was not observed in the virion RNAs of Rous-associated virus types 0 and 2 avian leukosis viruses and therefore is not packaged by all avian retroviruses. Since no other cellular message has been detected by this assay, it seems likely that the 34K mRNA found in 70S virion RNA is the result of selective packaging of an abundant host cell mRNA by certain avian retroviruses. (Author abstract) (34 Refs)

13. REPAIR OF LESIONS WHICH CAUSE PREMATURE TERMINATION OF TRANSCRIPTION IN CHICKEN EMBRYO CELLS IRRADIATED WITH ULTRAVIOLET LIGHT.

Hackett PB, Varmus HE, BishopJM

(c/o Bishop) Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143

Virology; 112(2):752-756 1981

The dose-response curves for the synthesis of rRNA and avian sarcoma virus mRNA, after irradiation of virus-infected chicken embryo fibroblast cells, have shoulders. We tested the hypothesis that the shoulders were the result of dark repair of transcription-terminating lesions after irradiation by measuring the amounts of rRNA synthesized at various times after the uv dose was applied. Our data support the hypothesis and indicate that the rate of repair in transcribed genes of chicken cells is about one or two lesions removed per 10(6) base pairs of DNA per min during the first 3 hr after irradiation. (Author abstract) (17 Refs)

14. THE GENESIS OF ROUS SARCOMA VIRUS MESSENGER RNAS.

Hackett PB, Varmus HE, Bishop JM

(c/o Bishop) Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143

Virology; 112(2):714-728 1981

Cells infected with Rous sarcoma virus (RSV) produce three classes of virus-specific mRNAs - one that has the size of the

viral genome and two that are smaller than the genome. We have explored the mechanisms by which these viral mRNAs are produced. The site for initiation of viral RNA synthesis was located by using irradiation with UV light to inactivate transcription in infected cells. The metabolism of viral RNA was elucidated by the use of glucosamine to effect satisfactory pulse-chase experiments. We conclude that transcription from the RSV provirus initiates at or near the nucleotide that encodes the 5' terminus of the viral genome. The primary transcript is subsequently processed to generate the subgenomic mRNAs, which appear independently of eath other between 15 and 45 min after the synthesis of their p recursor. Viral RNA has several distinctive fates: ca 35% remains in the cell in the form of the genomic length mRNA; 10-20% takes the form of subgenomic mRNAs; and the remaining 50% disappears from the cell, presumably because of export as viral genome (but perhaps also as a consequence of degradation within the cell). Our data provide a functional definition for the initiation of viral RNA synthesis and conform to the results of recent structural studies that have identified a possible 'promoter site' near the ends of the RSV provirus. (Author abstract) (66 Refs)

15. 5' TERMINAL NUCLEOTIDE NONCODING SEQUENCES OF RETROVIRUSES: A MODEL FOR EVOLUTIONARY DIVERGENCE (MEETING ABSTRACT).

Lovinger GG, Schochetman G

Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, MD

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 294 pp., 1981.

The 5' terminal noncoding nucleic acid sequences of a variety of mammalian type C retroviruses have been sequenced providing a useful evolutionary model for understanding how viral genomes diverge while preserving elements vital to their life cycles. The 5' terminal regions showed slowly divergent sequences with postulated replication, transcription, translation or integrative functions. Other sequences were interspersed which have diverged more rapidly. Based on computer assisted comparison of these two types of sequences, the following subgroups of mammalian type C viruses could be established: (1) viruses of murine (Mu) origin which include Rauscher-Mu leukemia virus (LV), Moloney-MuLV, Mo-MSV and gibbon ape LV, (2) viruses of old world monkey origin which include baboon endogenous virus and RD-114, and (3) viruses which include CPC-1 and MAC-1, two endogenous viruses of old world monkeys. Surprisingly, a distant relation in the third subgroup was shown to be Spleen necrosis virus, an exogenous avian virus belonging to the reticuloendotheliosis group. The intragroup relationships are defined by the relative number of correspondences within the rapidly divergent sequences. However, the intergroup relationships are preserved within the slowly divergent sequence. Sequence comparison of the 5' terminal regions of two other retroviruses, the type B mouse mammary tumor virus, and the unclassified equine infectious anemia virus showed homologies with the type C mammalian retroviruses only in the slowly divergent sequences. These conserved sequences help define common functional signals and point toward a common ancester for the retroviruses. (no Refs)

16. SELECTION OF RAT HEPATOMA CELLS DEFECTIVE IN HORMONE-REGULATED PRODUCTION OF MOUSE MAMMARY TUMOR VIRUS RNA.

Grove JR, Ringold GM

Dept. Pharmacology, Stanford Univ. Sch. Medicine, Stanford, CA, 94305

Proc Natl Acad Sci USA; 78(7,part2):4349-4353 1981

We have been studying the mechanism of glucocorticoid hormone action by using mouse mammary tumor virus (MMTV)-infected rat hepatoma cells as a model system. J2. 17, a clonal cell line that contains one MMTV provirus, induces tyrosine aminotransferase (TyrATase; L-tyrosine:2-oxoglutarate amino-transferase, EC2.6.1.5), viral RNA, and the cell surface viral glycoprotein gp52 in response to dexamethasone. Using a fluorescence-activated cell sorter and a rabbit antiserum directed against gp52, we selected a cell population that displays a reduced hormone-mediated increase in cell surface gp52. Fourteen clones of this population were assayed for induction of viral gp52 and RNA and of cellular TyrATase. The results of these assays revealed that the clones display a variety of responses to hormone. One clone has retained wild-type responses of both TyrATase and gp52. Six clones exhibit coordinately reduced or abolished responses of both markers. Seven clones show normal induction of TyrATase but reduced or undetectable induction of gp52. These latter clones exhibit reduced production of MMTV RNA and thus may represent a unique class of variants defective in the regulation of MMTV gene expression. (Author abstract) (36 Refs)

17. PROPERTIES OF A REPLICATION-DEFECTIVE MULV ISOLATED FROM CULTURED AKR LEUKEMIA CELLS (MEETING ABSTRACT).

Rein A, Lowy DR, Schultz AM, Gonda MA, Gerwin BI, Ruscetti SK, Bassin RH

Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, MD, 21701

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

We have described the isolation of a replication-defective ecotropic Murine leukemia virus (MuLV) from culture fluids of the AKRSL2 cell line. Using standard restriction mapping techniques, we have compared the genome of this defective MuLV to that of AKV. The two genomes are very similar to one another, but the defective genome contains an additional Kpn I site at 1.3 kbp and is 50 kbp shorter than AKV in the region between 1.8 and 3.0 kbp. Nonproducer cells containing the defective MuLV genome do not produce virus particles. They synthesize an env gene product, but no detectable gag or pol proteins. These cells thus provide a unique opportunity to study the properties of the env protein in the absence of other viral proteins. Metabolic studies show that gPr82env is processed in these cells to gp71 and p15(E) with essentially normal kinetics. Further, we find that the cells exhibit type-specific interference when challenged with ecotropic MSV pseudotypes. We conclude that (1) the absence of detectable gag or pol proteins is probably due to a frameshift or termination codon, at or slightly upstream from the novel Kpn I site at 1.3 kbp; (2) neither gag proteins nor particle synthesis are required for the pr imary cleavage event in env maturation; (3) viral interference is mediated solely by viral env proteins. Interference by env proteins in the absence of particle production may be an important natural defense against MuLV-induced disease. (1 Ref)

18. THE RELATIONSHIP BETWEEN GENOMIC RNAS OF POLYCYTHAEMIC FORMS OF SPLEEN FOCUS-FORMING FRIEND VIRUS AND ITS HELPER VIRUS.

Mol JN, Vonk WP, Pragnell IB, Stoof TJ
Dept. Genetics, Free Univ., De Boelelaan 1087, 1007MC Amsterdam, The Netherlands
J Gen Virol; 54(part2):367-377 1981

We have studied the relationship between Friend spleen focus-forming virus (SFFV) and its helper lymphoid leukemia virus (LLV) by comparing RNase T1 fingerprints of genomic RNAs. Our data indicate that about 70% of the SFFV sequence is a perfect copy of parts of the helper genome. We conclude that our SFFV and LLV isolates have co-evolved very closely and that SFFV-specific sequences are not identical in different Friend virus isolates. (Author abstract) (29 Refs)

19. FURTHER CHARACTERIZATION OF VIRUS-LIKE 30S (VL30) RNA OF MICE: INITIATION OF REVERSE TRANSCRIPTION AND INTRACELLULAR SYNTHESIS.

Dolberg D, Fan H
Lab. Chemical Biodynamics, Univ. California, Berkeley, CA, 94720
J Gen Virol; 54(part2):281-291 1981

We have studied the virus-like 30S (VL30) RNA sequences of mice. Previous work has shown that these sequences are coded in the mouse genome, expressed in some normal cells and released as pseudotypic particles from cells producing murine C-type retroviruses. VL30 sequences have some similarities to standard retrovirus RNA, but differences also exist. To further assess the similarities and differences, several aspects of VL30-specific metabolism were investigated. We studied the initiation of VL30-specific DNA synthesis during an endogenous reverse transcriptase reaction. Short initial VL30-specific cDNA transcripts were covalently attached to RNA as measured by equilibrium banding in cesium sulfate density gradients. Therefore, reverse transcription of VL30-specific cDNA is initiated by an RNA primer. The intracellular synthesis of VL30 RNA was investigated by pulse labeling uninfected JLS-V9 cells with 3H-uridine. Hybridization of the pulse-labeled nuclear RNA indicated that the major VL30-specific RNA evident after a 15 min label was the same size as the mature VL30 RNA. Thus, VL30 RNA is apparently not synthesized via a higher mol wt precursor. Both of these results demonstrate similarity of VL30 RNA sequences to standard retroviruses. One unique feature of VL30 RNA was detected. JLS-V9 cells contained both the monomeric VL30 RNA and a hydrogen-bonded 38S form which yielded the monomer when denatured. This contrasts with standard murine leukemia virus which is only found as a monomer within cells. (Author abstract) (31 Refs)

20. THE 28 S GENOMIC RNA OF AVIAN SARCOMA VIRUS PRCII CODES FOR THE TRANSFORMATION-SPECIFIC POLYPROTEIN P105.

DeLamarter JF, Neil JC, Ghysdael J, Vogt PK
(c/o Vogt) Dept. Microbiology, Univ. Southern California, Sch. Medicine, 2011 Zonal Ave., Los Angeles, CA, 90033
Virology; 112(2):757-761 1981

We have characterized the genomic RNA of the defective avian sarcoma virus PRCII. Replicating virus which consists of transforming PRCII and a nontransforming but replication-competent helper, PRCII-AV, contains two RNA species. One is identical in size to the 35 S genome of avian leukosis helper viruses. The second component migrates slightly faster than 28 S ribosomal RNA in polyacrylamide gel electrophoresis but cosediments with this RNA in sucrose gradients. In vitro translation across a gradient of velocity-sedimented poly(A)-containing PRCII virion RNA yielded three major proteins. The virion protein precursors Pr76gag and Pr180gag-pol were translated from 35 S RNA, while the transformation-specific polyprotein P105 was translated from 28 S RNA. P105 may be the only protein coded for by the PRCII genome, although this product would not exhaust the coding capacity of 28 S RNA. Whether translated in vitro or immunoprecipitated from transformed cells, P105 was essentially identical as demonstrated by comparative peptide maps. (Author abstract) (25 Refs)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

21. CHROMATOGRAPHIC EVIDENCE THAT THE AAA-CODING ISOACCEPTOR OF LYSINE TRNA PRIMES DNA SYNTHESIS IN MURINE MAMMARY TUMOR VIRUS.

Waters LC
Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
Virology; 112(2):766-769 1981

Most of the tRNA encapsulated within the murine mammary tumor virus is tRNAlys. The reversed-phase chromatographic pattern of tRNAlys isoacceptors in the viral-'free' tRNA and in the '70 S-associated' tRNA that is released at 65 degrees is similar to the pattern in virus-producing cells. However, the more tightly bound 70 S-associated tRNAlys is significantly enriched in the AAA-coding isoacceptor. This isoacceptor, but not the AAG-coding one, primes MuMTV 35 S RNA-directed DNA synthesis in vitro. (Author abstract) (16 Refs)

22. CLONAL ANALYSIS OF THE INTEGRATION AND EXPRESSION OF ENDOGENOUS AVIAN RETROVIRAL DNA ACQUIRED BY EXOGENOUS VIRAL INFECTION.

Humphries EH, Allen R, Glover C
Dept. Microbiology, Univ. Texas Health Science Center, Dallas, Dallas, TX, 75235
J Virol; 39(2):584-596 1981

Rous-associated virus-0 is one of several endogenous avian retroviruses that are transmitted vertically and that can be isolated from different inbred lines of chickens. These viruses, referred to here as induced-leukosis viruses bearing a subgroup E glycoprotein (ILV-E), are all closely related. Clonal populations of fibroblasts from line 15B and line 100 inbred chickens have been examined for the presence and expression of exogenously acquired ILV-E sequences. Restriction enzyme analysis of uniform populations of line 15B fibroblasts, prepared by cloning cells either before or after infection with ILV-E, indicates that viral sequences were inserted at multiple sites within the cell genome. Analysis of 49 clonal populations of line 100 fibroblasts containing between one and five copies of exogenous ILV-E sequences demonstrated that each clone was characterized by a unique set of viral DNA insertions within the cell genome. The expression of the exogenous ILV-E sequences within these fibroblast clones was examined by using reverse transcriptase activity as a measure of virus production. Some clones produced an amount of virus equivalent to that produced by an equal number of the uncloned ILV-E-infected parental fibroblasts. Other clones produced 5-to 10-fold less

virus. Still other clones produced no detectable virus at all. Among nine clones derived from cells containing a single copy of the ILV-E provirus, the level of virus production differed more than 100-fold. DNA from these clones was analyzed and several different restriction endonucleases to characterize the location and arrangement of the ILV-E sequences. All nine clones consisted of cells that appeared to contain a complete provirus inserted (i) in a different site within the cellular DNA and (ii) in an orientation that was colinear with the viral genomic RNA. It was observed that several cleavage sites potentially affected by methylation were equally available for cleavage in all clones regardless of the level of viral production. (Author abstract) (35 Refs)

23. A ROUS SARCOMA VIRUS PROVIRUS IS FLANKED BY SHORT DIRECT REPEATS OF A CELLULAR DNA SEQUENCE PRESENT IN ONLY ONE COPY PRIOR TO INTEGRATION.

Hughes SH, Mutschler A, Bishop JM, Varmus HE
Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724
Proc Natl Acad Sci USA; 78(7,part2):4299-4303 1981

The Rous sarcoma virus (RSV)-transformed rat cell line RSV-NRK-2 contains a single complete RSV provirus. We have obtained recombinant lambda clones that contain both ends of the RSV provirus and the flanking rat sequences. The provirus is integrated in unique DNA and is present in only one of the two homologous chromosomes. The rat sequences into which the RSV provirus integrated were also cloned from the RSV-NRK-2 cell line. The sequences of the regions involved in the recombination event have been determined and compared. Our data suggest that, compared with the sequence of viral DNA in the large circular form of unintegrated viral DNA, the provirus lacks two base pairs at each end and that the provirus is flanked by a six-base-pair direct repeat of cellular DNA. This six-base-pair repeat was appar ently created during the integration event because this sequence was present only once at the integration site before the provirus was inserted. A survey of eight other independent RSV transformed rat cell lines demonstrates that, in agreement with earlier results, the RSV proviruses have entered different segments of rat cell DNA. We have also determined the sequence of a second virus DNA-host cell DNA junction from a second RSV-transformed rat cell line (RSV-NRK-4) and find that there are no obvious similarities between the two integration sites or between the integration sites and the termini of viral DNA. (Author abstract) (32 Refs)

24. MOLECULAR CLONING OF THE AVIAN MYELOCYTOMATOSIS VIRUS GENOME AND RECOVERY OF INFECTIOUS VIRUSES BY TRANSFECTION OF CHICKEN CELLS.

Vennstrom B, Moscovici C, Goodman HM, Bishop JM
Dept. Microbiology, Biomedical Center, Uppsala, Sweden
J Virol; 39(2):625-631 1981

The avian retrovirus myelocytomatosis virus 19 (MCV) possesses an interesting diversity of oncogenic potentials, but the virus has proven difficult to study because of its inability to replicate without the assistance of a helper virus. We have therefore isolated and amplified the genome of MCV by molecular cloning in a prokaryotic vector. The topography of the cloned DNA was explored by the use of restriction endonucleases and radioactive complementary DNAs representing specific domains in avian retrovirus genomes. The cloned DNA appeared to be an authentic representation of the MCV genome: the size and genetic topography of the DNA were comparable to those of MCV, and transfection of the cloned DNA into chicken cells (in company with the DNA of a suitable helper virus) gave rise to virus with the genome and transforming potentials of MCV. The availability of cloned MCV DNA should facilitate a variety of genetic and biochemical manipulations directed at elucidating the mechanism of oncogenesis by MCV. (Author abstract) (25 Refs)

25. MOLECULAR CLONING AND PARTIAL CHARACTERIZATION OF UNINTEGRATED LINEAR DNA FROM GIBBON APE LEUKEMIA VIRUS.

Scott ML, McKereghan K, Kaplan HS, Fry KE
Cancer Biology Res. Lab., Dept. Radiology, Stanford Univ. Sch. Medicine, Stanford, CA, 94305
Proc Natl Acad Sci USA; 78(7,part2):4213-4217 1981

We have cloned the complete genome of an oncogenic primate retrovirus, the San Francisco isolate of gibbon ape leukemia virus, in a lambda phage vector. DNA sequence analysis and restriction endonuclease mapping of the inserted linear provirus demonstrated 9-base pair inverted repeats at its ends, flanking direct terminal repeats 470 base pairs in length. The (-) strong stop region of this DNA showed surprisingly low sequence homology to that of another gibbon ape leukemia virus isolate from an animal with similar disease. Analysis of the clone also revealed the terminal phosphate configuration of the linear pr ovirus. The recombinant phage is suitable for direct use as a hydridization probe to detect homologous retroviral sequences in human cell lines. (Author abstract) (28 Refs)

26. MAMMARY TUMOR VIRUS PROVIRAL DNA IN NORMAL MURINE TISSUE AND NON-VIRALLY INDUCED MAMMARY TUMORS.

Michalides R, Wagenaar E, Groner B, Hynes NE
Antoni van Leeuwenhoekhuis, 1066 CX Amsterdam, The Netherlands
J Virol; 39(2):367-376 1981

The Southern DNA filter transfer technique was used to study the involvement of the endogenous mouse mammary tumor virus (MMTV) in the development of mammary tumors of nonviral etiology. The presence of extra MMTV proviruses in the genomes of these non-virally induced mammary tumors would indicate an integration of the provirus of an activated endogenous MMTV. Acquisition of MMTV proviruses did not seem to be an absolute requirement for the development of hormone or carcinogenically induced mammary tumors in strain BALB/c nor for hormone-induced mammary tumors in mouse strains 020, C57BL, and C3Hf. In some hormone-induced mammary tumors we did observe extra MMTV proviruses in submolar quantities, indicating that reintegration may occasionally occur and that only a part of the tumor cells acquired new MMTV DNA information. Hormone-dependent and -independent primary mammary tumors of the mouse strain GR, which are controlled by the Mtv-2 mammary tumor induction gene, all acquired extra MMTV proviruses. Most of these extra MMTV proviral-DNA-containing fragments appeared present in submolar quantities, suggesting that only part of the tumor cells acquired extra MMTV proviral information. These findings indicate that the initially transformed mammary gland cells of non-virally induced mammary tumors do not necessarily acquire extra MMTV proviral DNA information, in contrast to the MMTV-induced mammary tumors, in which all tumor cells contain extra MMTV DNA information. (Author abstract) (34 Refs)

27. RECOMBINANT BACTERIOPHAGES CONTAINING THE INTEGRATED TRANSFORMING PROVIRUS OF GARDNER-ARNSTEIN FELINE SARCOMA VIRUS.

Fedele LA, Even J, Garon CF, Donner L, Sherr CJ
Viral Pathology Section, NCI, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 78(7,part2):4036-4040 1981

The integrated DNA provirus of the Gardner-Arnstein (GA) strain of feline sarcoma virus (FeSV) was molecularly cloned in a bacteriophage lambda vector. The cloned DNA fragment is 14.4 kilobase pairs long and contains a 6.7-kilobase provirus flanked by cellular sequences derived from nonproductively transformed mink cells. Transfection of mouse NIH/3T3 cells with the cloned DNA fragment induced foci of transformation at efficiencies of 10(4) focus-forming units/pmol of sarcoma virus DNA. Restriction endonuclease mapping and heteroduplex analyses were used to compare the GA-FeSV provirus with that of Sny der-Theilen (ST)-FeSV, a second strain that contains homologous transformation-specific sequences (v-fes). Both viruses have the general structure 5'-gag-fes-env-c region-3', each having retained portions of the feline leukemia virus (FeLV) gag and env genes. In addition to segments shared by the two sarcoma viruses, GA-FeSV contains 1.7 kilobases of extra sequences not found in ST-FeSV. Of these, at least 400-500 base pairs located near the 5' end of v-fes encode a portion of the GA-FeSV polyprotein; the remaining 1.2 kilobases are derived from the FeLV env gene but do not appear to encode any detectable product related to the FeLV envelope glycoprotein. The close homology of the v-fes sequences shows that GA- and ST-FeSV were formed by recombination of FeLV with similar portions of a cat cellular gene (c-fes). (Author abstract) (36 Refs)

28. TRANSFORMATION WITH SUBGENOMIC FRAGMENTS OF FELINE SARCOMA VIRUS PROVIRAL DNA.

Rosenberg ZF, Sahagan BG, Worley MB, Essex M, Haseltine WA
Dept. Microbiology, Harvard Sch. Public Health, Sidney Farber Cancer Inst., Boston, MA
Virology; 112(2):496-504 1981

Murine fibroblasts can be transformed by subgenomic fragments of feline sarcoma virus (FeSV) proviral DNA generated by BamH-I digestion of DNA of Snyder-Theilen FeSV and FeLV-infected mink cells. The efficiency of transformation of NIH 3T3 cells by BamH-I-treated FeSV proviral DNA was the same as that of untreated FeSV DNA. Focus-forming virus could not be rescued from the BamH-I-digested FeSV DNA-transformed cells. However, DNA from these transformed cells was able to induce transformation in secondary transfection experiments. Analysis of the DNAs from two BamH-I-digested FeSV DNA-transformed clones showed that the transformants contained only the 5' two-thirds of the FeSV provirus. Cells transformed by subgenomic fragments of FeSV DNA produce the 85,000-dalton gag-x protein, but do not express FOCMA. These experiments support the notion that the gag-x protein plays an active role in maintenance of the transformed phenotype. (Author abstract) (32 Refs)

29. CHARACTERIZATION OF MOUSE CELLULAR DEOXYRIBONUCLEIC ACID HOMOLOGOUS TO ABELSON MURINE LEUKEMIA VIRUS-SPECIFIC SEQUENCES.

Dale B, Ozanne B
Dept. Microbiology, Univ. Texas Health Science Center at Dallas, Dallas, TX, 75235
Mol Cell Biol; 1(8):731-742 1981

The nature of BALB/c-derived DNA sequences in the Abelson murine leukemia virus (A-MuLV) genome and their relationship to homologous sequences in mice (endogenous Abelson sequences) was examined. A DNA fragment of A-MuLV linear DNA was cloned into and Escherichia coli plasmid and used as a probe in Southern blot restriction enzyme analysis of high mol wt cellular DNAs prepared from mouse cells and cells of other species. Endogenous Abelson sequences are arranged similarly in all of the mouse strains examined. Other species possess nucleic acid sequences homologous to A-MuLV-specific sequences. The BALB/c-derived sequences as they appear in the virus are markedly different from the arrangement of homologous sequences in the mouse genome. No obvious rearrangement of endogenous Abelson sequences in the DNA of cells representing end-stage differentiation of the B-cell lineage or in thymocytes was observed. A map of normal mouse cellular sequences that are homologous to the A-MuLV probe is presented. (53 Refs)

30. ACCESSIBILITY OF SOME REGIONS OF CHROMATIN OF LEUKEMIC CHICKEN CELLS TO SINGLE STRAND NUCLEASE S1.

Leibovitch SA, Guillier M, Harel J
Laboratoire de Biologie Moleculaire et Physiopathologie Cellulaire, Institut Gustave-Roussy, G. R. N. 8 du CNRS, 16 bis, Avenue Paul Vaillant Couturier, 94800 Villejulf, France
Biochem Biophys Res Commun; 101(3):719-726 1981

Single strand nuclease S1 was used to probe the structure of DNA in Avian leukemic cells infected with Avian Myeloblastosis virus (AMV). Acid-soluble DNA fragments released after incubation of purified nuclei with 2000 units/ml (Aspergillus oryza) nuclease S1 appeared after a 5 min lag, with quantities increasing rapidly to approx 2% of total nuclear DNA at 20-30 min and to approx 4% after 4 hr. Control samples incubated without S1 released less than 1% of total DNA after 3 hr. Size distribution of the purified DNA was determined by agarose slab gel electrophoresis. Between 0-5 min of nuclease digestion only high mol wt (greater than 12,000 base pair) DNA fragments were obtained. Thereafter low mol wt segments appeared which could be resolved into at least the four size classes monomer through tetramer. A comparison of the effects of S1 nuclease and DNAase I on integrated AMV provirus sequences was made by hybridization of enzyme-treated nuclei to AMV-3HcDNA. Partial digestion of leukemic cell DNA with S1 nuclease did not significantly modify hybridization from the greater than 80% level found with non-nuclease-treated DNA. With DNAase I, however, hybridization of the virus-specific probe was reduced nearly to the 20% level obtained with normal embryonic chick cell DNA. S1 nuclease and DNAase I appear not to recognize the same sites in active chromatin regions. Furthermore, a limited number of chick leukemic cell DNA sites were susceptible to S1 nuclease. The resulting DNA fragments appearing as multiples of a monomeric unit were similar to fragments produced by micrococcal nuclease digestion. (18 Refs)

31. HYBRID PLASMIDS CARRYING PART OF THE ROUS SARCOMA VIRUS-SPECIFIC LEADER SEQUENCE.

Bromley PA, Voelimy R, Spahr PF

Dept. Molecular Biology, Univ. Geneva, 30, quai Ernest-Ansermet, 1211 Geneva 4, Switzerland

Biochim Biophys Acta; 655(1):41-48 1981

The 'strong-stop' DNA, complementary to the 5'-terminal 101 nucleotides of Rous sarcoma virus genomic RNA and subgenomic mRNAs can be used to isolate RSV-specific mRNA from infected cells. To analyze leader sequences of these mRNAs and to study the mechanism of their translation, relatively large quantities of strong-stop DNA are required. The construction of four plasmids carrying useful strong-stop DNA sequences for mRNA isolation is described. One such hybrid plasmid carries a DNA insertion containing sequences derived from a region starting 42 nucleotides from the 5'-end of RSV RNA, and the ending at the tRNATry primer attachment site. Since this plasmid lacks the 20-nucleotide terminal repeat sequence it can be used for the specific isolation of 5'-end fragments of RSV-specific mRNAs and of complementary DNA transcripts of these fragments. (Author abstract) (26 Refs)

CTHER RELATED STUDIES

32. VIRAL ONCOGENESIS BY PROMOTER INSERTION: AVIAN LEUKOSIS VIRUS DNA IS INTEGRATED NEXT TO THE CELLULAR MYC GENE IN 85% OF VIRALLY INDUCED LYMPHOMAS (MEETING ABSTRACT).

Astrin SM, Neel BG, Rogler C, Fang J, Skalka AM, Hayward WS

The Rockefeller University, New York, NY

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

We have analyzed the integrated proviruses in ALV induced lymphomas from 36 birds using virus-host junction fragments purified by molecular cloning. These junction fragments represent 2 of the 4 common tumor-specific integration sites previously reported. Using the cellular sequences in these clones as probes, we analyzed the integration sites in the tumors, the RNA in the tumors, and the organization of the inserted proviruses. Our data reveal that in 30 out of 36 tumors, integration has taken place within a 2 kilobase (kb) region upstream from the cellular myc gene. The integration events cluster in 4 regions, with the vast majority of the events occurring within a very limited region of about 200 base pairs. Data on the size of the proviral inserts suggests that the proviruses residing adjacent to myc are defective, often missing viral coding sequences as well as one of the two viral long terminal repeats (LTR) thought to encode a promoter for RNA polymerase II. The remaining LTR is oriented such that the promoter would read in the direction of the myc sequences. Data on the RNA species in the tumors are completely consistent with initiation within the viral promoter followed by transcription into the myc sequences. These findings are in agreement with the promoter insertion mechanism of oncogenesis previously proposed. (1 Ref)

33. MOLECULAR CLONING OF VIRUS-CELL JUNCTIONS FROM ALV-INDUCED LYMPHOMAS (MEETING ABSTRACT).

Neel BG, Rogler CE, Gasic GP, Skalka AM, Astrin SM, Hayward WS

The Rockefeller University, New York, NY

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Ha rbor Laboratory 294 pp., 1981.

In the present study, we have obtained molecular clones of the virus-cell junction fragments generated by digestion with Eco R1 from two independent avian leukosis viruses (ALV) induced lymphomas. Hybridization studies of the cloned DNAs confirm that in these tumors, proviral information is covalently linked to the c-myc gene. Proviral sequences are located upstream from c-myc and are oriented such that the viral promoter could be utilized for transcriptional activation of c-myc. In addition, by screening a total genomic library, we have isolated several clones containing the c-myc gene of a normal Rhode Island Red chicken. Comparison of the restriction maps of the tumor-derived clones with that of a c-myc clone has shown that they are co-linear. Thus, proviral integration in tumor occurs without gross rearrangement of c-myc sequences. Hybridization of MC29 RNA to restriction digests of one of the cloned c-myc fragments allows localization of myc coding sequences. Comparison of the hybridization pattern with known coding capacity of the v-myc gene strongly suggests that, consistent with the sequencing data of Gasic et al (abstract), c-myc exons are interrupted by at least one intron. (1 Ref)

34. NUCLEOTIDE SEQUENCE ANALYSIS OF MOLECULARLY CLONED VIRUS-CELL JUNCTION FRAGMENTS FROM ALV-INDUCED LYMPHOMAS (MEETING ABSTRACT).

Gasic GP, Neel BG, Rogler CE, Astrim SM, Hayward WS

The Rockefeller University, New York, NY, 10021

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

Molecularly cloned EcoR1 virus-cell junction fragments from different clonal avian leukosis virus (ALV) induced lymphomas, described by Neel et al (abstract), were sequenced. Preliminary sequence data for the two lymphoma virus-cell junction fragment inserts, designated BN-1 (tumor 7) and CR-1 (tumor 10) demonstrate that: 1) Based on the presence of U3-R-U5 sequences adjacent to the left EcoR1 site, BN-1 and CR-1 contain the large terminal redundancy (LTR) upstream from c-myc. 2) Other than U5 sequences, no viral leader information is contained in either clone. 3) A two nucleotide deletion at the virus-cell junction of BN-1 suggests that a normal proviral integration event has occured in this tumor. 4) On the basis of the last two points, the data suggests that the right LTR is present in these clones. 5) Downstream from the virus cell junction there exists an eight nucleotide sequence identical to the Adenovirus-2 Major Late first leader splice donor site. 5) Beyond this putative splice donor site, there exists an adenine thymine rich region characteristic of may introns. This data is consistent with the interpretation that 1) transcription initiates within the LTR, 2) the resulting transcript contains only R-U5 viral s equences, and 3) the primary transcript is spliced to generate the mature c-myc containing RNA. (1 Ref)

35. THE REV PROVIRUSES IN AVIAN BURSAL LYMPHOMAS: AMPLIFICATION OF THE PROVIRUS AND SPECIFIC INTEGRATION NEAR THE PROTO-ONCMCV LOCUS (MEETING ABSTRACT).

Noori-Daloii RM, Kung HJ, Crittenden LB, Witter RL

Department of Biochemistry, Michigan State University, East Lansing, MI

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

The proto-oncMCV gene (the cellular progenitor sequence of the oncogene of MC-29 virus) has recently been implicated in the induction of lymphoid leukosis (LL) in young chickens, following avian leukosis virus (ALV) infections. To study the specific involvement of the proto-oncMCV locus in avian lymphomagenesis, we wished to analyze chicken B-lymphoma tumors induced by agents other than ALV. We exploited our previous findings that the helper-independent, chicken syncitia viruses (CSV; a member of the reticuloendotheliosis virus (REV) family) - genetically unrelated to ALV - are also capable of inducing LL in chickens with similar incidence and pathology. We have characterized the structure of the REV proviruses in the induced tumors and report here that the proviral DNA is integrated next to the proto-oncMCV in every tumor analyzed. This data is compatible with our earlier findings that ALV proviruses are linked to the proto-oncMCV genes in ALV induced tumors and lends further credence to the hypothesis that proto-oncMCV and its adjacent sequences are important in lymphocyte transformation. We also obtained evidence which indicate amplifications of the chromosome region encompassing the CSV provirus and proto-oncMCV locus have occured in some of the tumors. (no Refs)

36. IDENTIFICATION OF THE POSSIBLE ONCOGENES INVOLVED IN DIFFERENT NEOPLASMS INDUCED BY AVIAN LEUKOSIS VIRUS (MEETING ABSTRACT).

Fung YK, Crittenden LB, Kung HJ, Fadly AM

Department of Biochemistry, Michigan State University, East Lansing, MI, 48824

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

Avian leukosis viruses (ALV) are known for their ability to induce lymphoid leukosis in young chickens. These viruses, however, are also able to induce a number of other neoplasms in chickens at a lower frequency. Since ALV genome does not appear to encode an oncogene, it was proposed that the viruses induce neoplastic transformation of the target cell by activation of a cellular oncogene. To examine this possibility, we have analyzed the ALV provirus structure in several different types of tumors induced by ALV. The results are consistent with the notion that viral DNA integrates next to a specific cellular 'oncogene' which then triggers a particular type of neoplastic transformation. In the erythroblastosis tumor, we found that the ALV provirus is integrated next to the cellular sequences related to the oncogene of avian erythroblastosis virus (AEV). Avian myelocytomatosis virus (MC29) is capable of inducing nephroblastoma in chicks. In ALV induced nephroblastoma, we found the ALV provirus is linked to the cellular sequence related to the oncogene of MC29. We have demonstrated linkage of RAV-1 proviruses to the cellular sequences related to MC29 in most of the lymphoid bursal tumors. (no Refs)

37. TWO DISTINCT CANDIDATE TRANSFORMING GENES OF LYMPHOID LEUKOSIS VIRUS-INDUCED NEOPLASMS.

Cooper GM, Neiman PE

Sidney Farber Cancer Inst., Harvard Medical Sch., Boston, MA, 02115

Nature (Lond); 292(5826):857-858 1981

Further analysis of the avian lymphoid leukosis virus (LLV)-induced tumors used as DNA donors in transfection experiments with NIH 3T3 mouse cells confirmed that the tumors contained exogenous DNA long terminal redundancy (LTR) sequences near the cellular gene (c-myc) homologous to the presumptive transforming gene of myelocytomatosis virus strain MC29. However, chicken c-myc sequences were not detected in NIH 3T3 cells transformed by the DNAs of LLV-induced bursal tumors or a virus-induced lymphoma. These results suggested that LLV-induced tumors contain at least two genes with potential oncogenic activity: a c-myc gene activated by integration of a viral LTR and a distinct cellular gene capable of transforming NIH 373 cells. (12 Refs)

38. ACTIVATION OF C-MYC IN THE VICINITY OF THE INTEGRATION SITE OF AN EXOGENOUS PROVIRUS IN A CELL LINE DERIVED FROM AN AVIAN LEUKOSIS VIRUS (ALV) INDUCED BURSAL LYMPHOMA (MEETING ABSTRACT).

Schubach WH, Groudine M, Neiman PE

Fred Hutchinson Cancer Research Center, Seattle, WA

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

LSCC-RP-9 (RP-9) is a B cell line derived from a bursal lymphoma which contains at least two activated transforming genes potentially responsible for the malignant properties of these tumor cells. The first is the cellular analogue of the transforming gene of the acute leukemia virus MC-29, called c-myc, which is closely linked to an ALV long terminal repeat (LTR) in RP-9 and most ALV induced lymphoma. The second gene transforms NIH/3T3 mouse cells in transfection assays, and is not closley linked to ALV sequence. To investigate the mechanism of activation of such genes we have studied the structure, nucleosome organization and transcriptional activity of the region around the activated c-myc in RP-9 cells using genomic DNA clones for the relevant viral and cellular sequences. We have observed that the pattern of nucleosome sensitivity to DNAse I is altered in the region and that, in addition to the constituative messenger RNA (mRNA) c-myc species, there are at least four new and abundant c-myc mRNAs terminating at or near the 3' boundary of the gene. None of these steady state mRNAs bear viral LTR sequences. Furthermore, the presumptive viral promoter 7kbp from the 5' end of c-myc is oriented in the opposite direction for activation of transcription into the c-myc. We suggest the ALV induced transcriptional activation of c-myc in these cells is not mediated by 'downstream promotion' in these cells, and that regional chromosomal activation by as yet unclear mechanisms may explain the activity of these transforming genes. This notion is supported by the observations regarding the transcription and alteration of the chromatin structure of c-myc in avian cells transformed by mechanisms other than retroviruses as well as in different compartments of the hematopoietic lineage. (no Refs)

39. ACTIVATION OF RELATED TRANSFORMING GENES IN MOUSE AND HUMAN MAMMARY CARCINOMAS (MEETING ABSTRACT).

Lane MA, Sainten A, Cooper GM

Department of Pathology, Sidney Farber Cancer Institute, Boston, MA, 02115

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 294 pp., 1981.

High mol wt DNAs of five mouse mammary tumor virus (MMTV)-induced tumors, two chemical carcinogen-induced mouse mammary tumors, and one human mammary tumor cell line (MCF-7) were assayed for the presence of transmissible activated transforming genes by transfection of NIH 3T3 mouse cells. DNAs of all 5 MMTV-induced tumors, one chemical carcinogen-induced tumor, and the human tumor cell line induced transformation with high efficiencies. NIH cells transformed by DNAs of MMTV-induced tumors did not contain exogenous MMTV DNA sequences, indicating that MMTV-induced mammary carcinomas contained activated cellular transforming genes that were not linked to viral DNA, NIH cells transformed by DNA of the human tumor cell line contained human DNA sequences detectable by hybridization with human repetitive DNA. The transforming activities of DNAs of all five MMTV-induced tumors, the chemical carcinogen-induced mouse tumor, and the human tumor cell line were inactivated by digestion with the restriction endonucleases Pvu II and Sac I, but not by Bam HI, Eco RI, Hind III, Kpn I or Xho I. These results indicate that the same or closely related transforming genes were activated in six different mouse mammary carcinomas, induced by either MMTV or a chemical carcinogen, and in a humam mammary carcinoma cell line. (no Refs)

40. ANALYSIS OF SEQUENCES IN HUMAN DNA RELATED TO THE TRANSFORMING GENES OF MURINE TRANSFORMING VIRUSES (MEETING ABSTRACT).

Prakash K, Tronick SR, Eva A, Devare S, Reddy EP, Srinivasan A, Aaronson SA

National Cancer Institute, Bethesda, MD

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Ha rbor Laboratory 294 pp., 1981.

Using cloned DNA probes specific for the transforming regions of Moloney murine sarcoma virus (M-MSV) and Abelson murine leukemia virus (A-MuLV), we have detected homologous sequences in human DNA. The M-MSV (mos) probe revealed a single 2.5 kbp band in Eco RI-cleaved DNA, whereas two bands (15 and 4 kbp) were detected using the A-MuLU (abl) probe. These viral transforming gene-related sequences were cloned from human DNAs either by standard gene enrichment techniques or by screening cloned human DNA libraries. We have performed structural comparisons of viral and normal mouse cellular mos and abl sequences with their human homologues by utilizing restriction enzyme mapping and heteroduplex techniques. The cloned human DNA probes for the mos and abl related sequences and for their respective flanking regions were also utilized in order to study the expression and topography of their related transcripts in human cells. (no Refs)

41. HUMAN TUMOR CELLS CONTAIN TRANSCRIPTS RELATED TO THE TRANSFORMING GENES OF RETROVIRUSES (MEETING ABSTRACT).

Eva A, Robbins K, Andersen P, Srinivasan A, Papas T, Tronick S, Reddy EP, Westin E, Wong-Staal F, Gallo R, Ellmore N, Aaronson S

Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 294 pp., 1981.

We have molecularly cloned the genomes of a number of transforming retroviruses. Their transforming genes are evolutionarily conserved in the DNA of normal vertebrates, including human. We have utilized viral transforming genes as molecular probes to search for homologous RNA transcripts in human tumor cell lines. These lines exhibit phenotypic properties of the orginal tumors, and reproduce the same histopathology in nude mice. Northern blotting analysis of poly:A-containing RNAs from human sarcoma, melanoma and carcinoma derived cell lines revealed multiple RNA species that hybridized with probes specific for the transforming genes of BALB-mouse sarcoma virus, Abelson-murine leukemia virus and MC29 virus. In contrast, a probe specific for the transforming gene of simian sarcoma virus detected a single transcript in sarcoma derived tumor cell lines. Transcripts related to the transforming genes of some of these viruses were also detected in normal human fibroblasts. We conclude that RNAs related to the transforming genes of certain transforming retroviruses are frequently detectable in human cells. (no Refs)

42. ANALYSIS OF MULV PROVIRUSES IN THE AKR MOUSE DURING THE DEVELOPMENT OF LYMPHOMAS USING CLONED DNA VIRAL SPECIFIC PROBES (MEETING ABSTRACT).

Yoshimura F, Breda M

Fred Hutchinson Cancer Research Center, Seattle, WA, 98104

RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Ha rbor Laboratory 294 pp., 1981.

We have used molecular clones of two of the viruses implicated in leukemogenesis in the AKR mouse. One clone is of the AKR ecotropic Murine leukemia virus (MuLV) (clone 623). The other is a lambda clone which we have prepared of one of the recombinant MCF viruses, MCF-13, by using closed circular DNAs from recently infected cells. We have derived a detailed restriction map of MCF-13 MuLV by using Hirt linear viral DNA and this cloned DNA and show that different cleavage sites, eg, Eco RI, are present mainly in the envelope region of MCF-13 compared with AKR MuLV. Fragments from the envelope region of the AKR and MCF-13 MuLV clones have been isolated, characterized to be specific for each virus, and used to analyze tissue DNAs from control or untransformed and thymoma DNAs. By using the AKR MuLV specific probe we observed three basic patterns of Eco RI fragments from both untransformed and transformed tissue DNAs. These Eco Ri fragments are found in different combinations in DNAs taken from different animals, suggesting a polymorphism among individuals in the AKR/J population used in these studies. We failed to detect any amplification of these AKR MuLV specific sequences in six different thymomas. By using either MCF-13 MuLV cyclic DNA or the DNA specific probe, on the other hand, we have detected amplified DNA fragments in the thymomas of AKR animals injected with this virus at birth. These extra fragments are found in low copy number. (no Refs)

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VIRAL PROTEINS

1. EVIDENCE FOR TWO FORMS OF REVERSE TRANSCRIPTASE IN HUMAN PLACENTA OF A PATIENT WITH BREAST CANCER. PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF THE ENZYMES.

Vogel A, Chandra P
(c/o Chandra) Zentrum der Biologischen Chemie, Abteilung fur Molekularbiologie der Universitat Frankfurt/Main, Theodor-Stern-Kai 7, D-6000 Frankfurt (Main) 70, W. Germany

Biochem J; 197(3):553-563 1981

Two DNA polymerases with properties of viral RNA-directed DNA polymerase were found in the placenta of a patient with breast cancer. Both enzyme activities were purified by column-chromatographic procedures or by preparative isoelectric focusing. The most distinguishing feature of the two enzymes is their specificity to transcribe (rA)n.(dT)12 or (rC)n.(dG)18. The two enzymes differ with respect to their elution profiles from the phosphocellulose column, isoelectric point, mol wt, bivalent-cation requirements and thermal stability. Serological analysis of the (rA)n.(dT)12-activated enzyme

showed that this enzyme is immunologicaly not related to DNA polymerase-gamma, or to any of the reverse transcriptases purified from retroviruses of avian, murine and subprimate origin. However, the activity of this enzyme was neutralized by antibodies to reverse transcriptase purified from human spleen of a patient with myelofibrosis. Attempts to purify reverse transcriptase from normal human placenta were repeatedly unsuccessful. Once the crude homogenate of normal human placenta was freed from endogenous nucleic acids, no (rC)n.(dG)18-dependent activity could be detected. (Author abstract) (17 Refs)

2. PHOSPHORYLATION OF THE ABELSON MURINE LEUKEMIA VIRUS TRANSFORMING PROTEIN.

Witte ON, Ponticelli A, Gifford A, Baltimore D, Rosenberg N, Elder J
Dept. Microbiology, Molecular Biology Inst., Univ. California, Los Angeles, CA, 90024
J Virol; 39(3):870-878 1981

The Abelson murine leukemia virus transforming gene product is a phosphorylated protein encoded by both viral and cellular sequences. This gene product has an amino-terminal region derived from the gag gene of its parent virus, and a carboxyl-terminal region (abl) derived from a normal murine cellular gene. Using a combination of partial proteolytic cleavage techniques and antisera specific for gag and abl sequences, we mapped in vivo phosphorylation sites to different regions of the protein. Phosphoproteins encoded by strain variants and transformation-defective mutants of Abelson murine leukemia virus with defined deletions in the primary sequence of abl region were compared by two-dimensional limit digest peptide mapping. Specific phosphorylation pattern differences for wild-type and mutant proteins probably represented deletions of specific phosphate acceptor sites in the abl region. An in vitro autophosphorylation activity copurified with the Abelson murine leukemia virus protein from transformation-competent strains. A peptide analysis of such in vitro reactions demonstrated that these phosphorylation sites were restricted to the amino-terminal region, and the specific sites appeared to be unrelated to the sites found on proteins phosphorylated in vivo. Thus, the autophosphorylation reaction probably correlates with an activity important in transformation, but the specific end product in vitro bears little resemblance to its function in vivo. (Author abstract) (34 Refs)

3. IN VITRO PROTEOLYTIC CLEAVAGE OF GAZDAR MURINE SARCOMA VIRUS P65GAG.

Maxwell S, Arlinghaus RB
Dept. Tumor Virology, Univ. Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
J Virol; 39(3):963-967 1981

Moloney murine leukemia virus, disrupted in concentrations of 0.1 to 0.5% Nonidet P-40, catalyzed the cleavage of p65, the gag gene polyprotein of the Gazdar strain of murine sarcoma virus, into polypeptides with sizes and antigenic determinants of murine leukemia virus-specified p30, p15, pp12, and p10. Cleavage performed in the presence of 0.15% Nonidet P-40 in water yielded polypeptides of approx 40,000 (P40) and 25,000 (P25) Mr. In vitro cleavage performed in a buffered soln containing dithiothreitol in addition to 0.1% Nonidet P-40 allowed the efficient processing of P40 to p30 and a band migrating with p10. Immunoprecipitation with monospecific sera indicated that P40 contained p30 and p10, whereas P25 contained p15 and pp12 determinants. P40 and P25 are similar in size and antigenic properties to Pr40gag and Pr25gag observed in infected cells. (Author abstract) (16 Refs)

4. BIOCHEMICAL ANALYSIS OF THE P30'S OF N-, B-, AND B--NB-TROPIC MURINE LEUKEMIA VIRUSES OF BALB/C ORIGIN.

Schindler J, Gautsch JW, Lerner RA, Hopkins N
Ortho Pharmaceutical Corp., Raritan, NJ, 08869
J Virol; 39(3):703-712 1981

Previous analysis of the virion proteins of an N- and a B-tropic Type C virus of BALB/c mice, of 16 N-tropic recombinants (XLPN viruses) between these viruses, and of eight NB-tropic viruses derived from the B-tropic virus suggested that among these closely related viruses N-, B-, or NB-tropism was associated with the electrophoretic mobility of p30 on sodium dodecyl sulfate-polyacrylamide gels, and thus that p30 might determine this phenotype. To obtain further evidence for the association of structural markers of p30 with N-, B-, or NB-tropism, we have analyzed the p30's of these same viruses by using two-dimensional tryptic peptide mapping and slab gel isoelectric focusing. The results of these analyses suggest that (i) a single peptide unique to the N-tropic virus p30 is present in the p30 of all N-tropic recombinants; (ii) a single peptide unique to the B virus p30 is not present in p30's of the N-tropic recombinants, and this peptide is also absent in p30's of NB-tropic viruses derived from the B-tropic virus; and (iii) p30's of NB-tropic viruses possess a new tryptic peptide not found in the p30 of their B-tropic virus progenitors, and this new peptide is not found in the p30 of the N-tropic virus of BALB/c or the XLPN viruses. These results are consistent with the possibility that p30 may determine the N-, B-, or NB-tropism of murine leukemia viruses. In addition, these studies indicate that some of the N-tropic recombinants have experienced recombination within the p30 gene. (Author abstract) (18 Refs)

5. DEMONSTRATION OF AVIAN SARCOMA VIRUS-CODED PP60SRC IN VIVO AND THE ANTI-PP60SRC IMMUNE RESPONSE IN CHICKENS.

Ellwart-Tschurtz M, Kurth R
Friedrich Meischer-Laboratorium, Max Planck-Gesellschaft, 7400 Tubingen, W. Germany
J Virol; 39(3):950-953 1981

The avian sarcoma virus (ASV)-coded transforming protein pp60src was originally detected in vitro in ASV-transformed avian and mammalian cells in experiments involving mammalian antisera to ASV-induced tumors. It is demonstrated here that pp60src is also expressed in vivo in ASV tumors of chickens. Furthermore, the existence of the endogenous pp60src in all chicken cells does not impair the immune response to exogenous pp60src in the chicken. Whereas chicken antibodies can bind to pp60src, they do not serve as substrates for the protein kinase activity of this transforming protein. (Author abstract) (8 Refs)

6. TWO CELLULAR PROTEINS THAT IMMUNOPRECIPITATE WITH THE TRANSFORMING PROTEIN OF ROUS SARCOMA VIRUS.

Oppermann H, Levinson AD, Levintow L, Varmus HE, Bishop JM, Kawai S
Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143
Virology; 113(2):736-751 1981

The transforming gene (src) of Rous sarcoma virus encodes a 60,000-dalton phosphoprotein (pp60src) with the ability to phosphorylate tyrosine in certain protein substrates. The enzymatic activity of pp60src is thought to mediate neoplastic

transformation by src. It would therefore be useful to identify cellular proteins that interact with pp60src on the chance that these proteins might be substrates for the kinase activity of the viral protein or be otherwise involved in neoplastic transformation of the host cell. In pursuit of this objective, we characterized the proteins that coprecipitate with pp60src in immune complexes. These proteins proved to be of two types. (i) Most immune complexes contained a series of proteins (50,000 to 58,000 daltons) that were apparently derived from pp60src by sequential degradation from the amino terminus. (ii) Two proteins (50,000 and 89,000 daltons) coprecipitated with pp60src, probably by virtue of their ability to bind to the viral protein. We used temperature-sensitive mutants in src to explore the possibility that the two cellular proteins might be substrates for the protein kinase activity of pp60src: propagation of infected cells at the nonpermissive temperature failed to affect the phosphorylation of either of the proteins. We conclude that at least two cellular proteins are associated with pp60src prior to immunoprecipitation with antisera directed against the viral protein. It is possible that neither of these proteins is a substrate for the protein kinase activity of pp60src, however, and their role in neoplastic transformation by src (if any) remains moot. (38 Refs)

7. CLONAL ISOLATE OF THE SIMIAN SARCOMA VIRUS CODES FOR A GAG-RELATED 65,000-DALTON PROTEIN.

Thiel HJ, Matthews TJ, Broughton EM, Weinhold KJ, Bolognesi DP, Graf T, Beug H
Bundesforschungsanstalt fur Viruskrankeiten der Tiere, P.O. Box 1149 Paul Ehrlich-Str. 28, 74 Tubingen, W. Germany
Virology; 114(1):124-131 1981

An autologous antiserum against SSV nonproducer (SSV-NP cells) recognizes a 65,0 00-dalton protein (p65). This molecule does not appear to exhibit transformation-specific properties, but is serologically and biochemically very similar to the SSAV gag-precursor pr65gag. Since p65 appears to be cleaved upon superinfection of SSV-NP cells with SSAV, it may provide a unique model to study gag-precursor cleavage of mammalian C-type viruses. (Author abstract) (23 Refs)

8. SYNTHESIS OF VIRUS-SPECIFIC PROTEINS IN SIMIAN SARCOMA VIRUS-TRANSFORMED PRIMATE CELLS.

Bergholz CM
Paper Product Div., Human Safety Support, Proctor and Gamble Co., 6100 Center Hill Road, Cincinnati, OH, 45224
Virology; 114(1):113-123 1981

Marmoset monkey cells productively and nonproductively transformed by simian sarcoma virus (SSV) were evaluated for expression of simian sarcoma associated virus (SSAV)-specific structural polypeptides and SSV transformation-specific polypeptides. Virus-producing cells contained, in addition to virus structural proteins, a 62,000-dalton polypeptide (pr62 gag) precipitable by antisera specific for SSAV p28 or p10, and a polypeptide of approx 80,000 daltons (pr80env) which could be glucosamine-labeled and which was precipitated by antisera specific for SSAV envelope proteins. Results of pulse-chase experiments provided evidence for a precursor-product relationship between pr62 gag and p28 and between pr80 env and the envelope proteins gp70, p15E, and p12E. Pr62 and p12 were the only detectable phosphorylated virus-specific polypeptides. Nucleic acid hybridization techniques demonstrated SSAV-related DNA and RNA in clonal SSV-transformed nonproducer marmoset cell lines (HF/SSV-NP), but synthesis of SSAV-related protein was demonstrated in only one of five of these lines. This cell line expressed pr62 which was not processed to yield p28 or other gag gene products. Gel electrophoresis of immunoprecipitates demonstrated that SSV does not express a transformation-specific polyprotein comprised in part of either p28, p10, or p12 and a nonstructural component. Antibodies which precipitated a protein specific for SSV-transformed cells were not detected in sera from marmoset monkeys bearing SSV-induced tumors, or in sera from a goat or marmosets inoculated with autologous SSV-transformed cells or HF/SSV-NP cells. (Author abstract) (33 Refs)

VIRAL RNA

9. CONSTRUCTION AND ISOLATION OF A TRANSMISSIBLE RETROVIRUS CONTAINING THE SRC GENE OF HARVEY MURINE SARCOMA VIRUS AND THE THYMIDINE KINASE GENE OF HERPES SIMPLEX VIRUS TYPE 1.

Wei CM, Gibson M, Spear PG, Scolnick EM
Lab. Tumor Virus Genetics, NCI, Bethesda, MD, 20205
J Virol; 39(3):935-944 1981

We constructed lambda recombinants containing the Harvey murine sarcoma virus genome and the thymidine kinase (tk) gene of herpes simplex virus Type 1 linked to each other. The tk gene was located in a position downstream from both the long terminal repeat and the src gene of Harvey murine sarcoma virus. The DNAs of the lambda recombinants were used to transfect NIH3T3 mouse fibroblasts in order to obtain Harvey murine sarcoma virus DNA-induced foci of transformed cells. The transformed foci were superinfected with a helper-independent retrovirus, and new individual retroviruses were isolated from the superinfected foci. The new viruses could induce focus formation on NIH3T3 cells and could convert NIH3T3(TK-) cells into TK+ cells by carrying the herpes simplex virus Type 1 tk gene into the TK- cells. From virus-infected cells, we isolated nonproducer foci on NIH3T3 cells and TK+ transformants on NIH3T3(TK-) cells containing one such new viral genome coding for the dual properties. The new retroviral sequence in the nonproducer cells could be rescued into virus particles at high titers by superinfection with a helper-independent retrovirus. A hybridization analysis indicated that the recombinant virus contained both the Harvey murine sarcoma virus src sequence and the tk gene sequence in a single RNA species approx 4.9 kilobases long. We concluded that retroviruses can be used as true vectors for genes other than genes that lead to oncogenesis. (Author abstract) (50 Refs)

10. CHARACTERIZATION OF TRANSFORMATION- AND REPLICATION-SPECIFIC SEQUENCES OF RETICULOENDOTHELIOSIS VIRUS.

Cohen RS, Wong TC, Lai MM
(c/o Lai) Dept. Microbiology, Univ. Southern California Sch. Medicine, Los Angeles, CA, 90033
Virology; 113(2):672-685 1981

Using a combination of hybridization and oligonucleotide fingerprinting techniques we have determined the sequence relatedness of oncogenic avian reticuloendotheliosis virus (REV) to its associated helper virus, REV-A, and to the other members of the reticuloendotheliosis (RE) virus group. Our studies have shown that approx 30% of the genomic sequences of REV are unrelated to the nononcogenic RE viruses. These

REV-specific, and presumably oncogenic, sequences are arranged in a contiguous fashion, possibly interrupted by a short stretch of REV-A-related sequences, localized between 1 and 2.7 kilobases (kb) from the 3'-end on the REV genome. We have also identified two regions of REV-A sequences which are deleted in the REV genome. The first region encompasses 3 kb of sequences in the 5'-half of the genome, presumably corresponding to the gag-pol genes. The second region represents 1.5(kb) of the env sequences. These deletions could account for the genetic defectiveness of REV. By studying the sequence relatedness between REV-A and the other RE viruses, we have shown that REV is the only RE virus which contains these oncogenic sequences. We have also determined the relatedness between these viruses. In addition, we have identified a hypervariable region which maps in or near the env gene. The possible significance of this region in accounting for both the varied pathogenicity of the RE viruses and the origin of oncogenic REV is discussed. (Author abstract) (39 Refs)

11. GENOMES OF MURINE LEUKEMIA VIRUSES ISOLATED FROM WILD MICE.

Chattopadhyay SK, Oliff AI, Linemeyer DL, Lander MR, Lowy DR
Dermatology Branch, Pediatric Oncology Branch, NCI, Bethesda, MD, 20205
J Virol; 39(3):777-791 1981

The genomes of murine leukemia viruses (MuLV) isolated from wild mice have been studied. Detailed restriction endonuclease maps of the 8.8-kilobase (kb) unintegrated linear viral DNAs were derived for five ecotropic and five amphotropic MuLV's from California field mice, for Friend MuLV, and for one ecotropic and one xenotropic MuLV from Mus musculus castaneus. In general, the California MuLV's were similar in their leftward 6 kb (corresponding to the leftward long terminal repeat [LTR], gag, and pol) and rightward 1 kb (7.8 to 8.8 kb, corresponding to p15E and the rightward LTR). For the region spanning 6.0 to 7.7 kb (which includes the sequences that encode gp70) the amphotropic MuLV's shared few enzyme sites with the ecotropic MuLV's, although the California ecotropic MuLV were highly related to each other in this region, as were the amphotropic MuLV's. Cross-hybridization studies between amphotropic and California ecotropic MuLV DNAs indicated that they were not homologous in the region 6.3 to 7.6 kb; the California ecotropic viral DNAs cross-hybridized in this region to AKR ecotropic MuLV. When the California viral DNAs were compared with AKR ecotropic viral DNA, many differences in enzyme sites were noted throughout the genome. The U3 regions of the wild mouse LTRs showed partial homology to this region in AKR MuLV. The LTR of Moloney MuLV was highly related to that of the California MuLV's, whereas the LTR of Friend MuLV appeared to be a recombinant between the two types of LTRs. The M musculus castaneus isolates were most closely related to ecotropic and xenotropic MuLV's isolated from inbred mice. One amphotropic MuLV DNA was cloned from supercoiled viral DNA at its unique EcoRI site in pBR322. Viral DNAs with one and two LTRs were isolated. After digestion with EcoRI, DNAs of both types were infectious. It is concluded that ecotropic and amphotropic MuLVs differ primarily in the region which encodes gp70. (Author abstract) (48 Refs)

12. ORIGIN OF MINK CYTOPATHIC FOCUS-FORMING (MCF) VIRUSES: COMPARISON WITH ECOTROPIC AND XENOTROPIC MURINE LEUKEMIA VIRUS GENOMES.

Chattopadhyay SK, Lander MR, Gupta S, Rands E, Lowy DR
Bldg. 10, Room 2B50, NIH, Bethesda, MD, 20205
Virology; 113(2):465-483 1981

Restriction endonuclease maps have been developed for the viral DNAs from nine xenotropic and eleven MCF murine leukemia viruses (MuLV) isolated from AKR and other mouse strains. In contrast to the highly related nature of ecotropic viral DNAs isolated from inbred mice and from M m molossinus, each xenotropic and MCF viral DNA was unique. Xenotropic MuLV DNAs could be divided into two classes, which correlated with previously reported serological and biochemical data; one xenotropic MuLV isolated from an AKR mouse showed features of both classes. Ecotropic viral DNA hybridized poorly or not at all to a 1.2 kbp segment of xenotropic viral DNA located in env 6.3-7.5 kbp from the left end of the viral DNAs. All MCF viral DNAs contained nonecotropic sequences in a portion of the env gene region, but some MCF viruses were composed principally of nonecotropic sequences. The nonecotropic regions of the MCF viral DNAs were related to xenotropic MuLV DNA, but many MCF viral DNAs contained sequences not found either in xenotropic or ecotropic MuLV DNA. It was concluded that these MCF viruses probably arose via recombination between ecotropic MuLV and endogenous MuLV DNA sequences; sequences of recombination include a portion of env, but need not be limited to this region. The polytropic host range of MCF viruses may represent an endogenous viral function. (Author abstract) (54 Refs)

13. NUCLEIC ACID HOMOLOGY BETWEEN AVIAN AND MAMMALIAN TYPE C VIRUSES: RELATEDNESS OF RETICULOENDOTHELIOSIS VIRUS CDNA TO CLONED PROVIRAL DNA OF THE ENDOGENOUS COLOBUS VIRUS CPC-1.

Rice NR, Bonner TI, Gilden RV
Biological Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD, 21701
Virology; 114(1):286-290 1981

Reverse transcriptase and p30 of the reticuloendotheliosis viruses (REV), which are Type C viruses horizontally transmitted among certain avian species, have previously been shown to be related to mammalian rather than to other avian leukosis/sarcoma Type C viral proteins. Though the closest homology has been seen between proteins of REV and those of the endogenous macaque viruses (MAC-1 and MMC-1), relatedness of their nucleic acids cannot be detected by routine liquid hybridization. We report here that nucleic acid homology between REV and the colobus monkey endogenous virus (CPC-1), which is highly related to the macaque viruses, can be demonstrated when hybridization is carried out with highly labeled [32P]cDNA and cloned viral DNA immobilized on nitrocellulose. Related regions occur within both the pol and the gag genes of the colobus viral genome. Thus, the REV family of viruses appears to be descended from an ancestral virus which also gave rise to the MAC-1, MMC-1, CPC-1 group of viruses now endogenous in primates. (Author abstract) (20 Refs)

14. GENERATION OF TRANSFORMING VIRUSES IN CULTURES OF CHICKEN FIBROBLASTS INFECTED WITH AN AVIAN LEUKOSIS VIRUS.

Stavnezer E, Gerhard DS, Binari RC, Balazs I
Genetics and Molecular Biology Unit, Graduate Sch., Sloan-Kettering Inst. for Ca ncer Res., New York, NY, 10021
J Virol; 39(3):920-934 1981

During serial passages of an avian leukosis virus (the transformation-defective, src deletion mutant of Bratislava 77 avian sarcoma virus, designated tdB77) in chicken embryo fibroblasts, viruses which transformed chicken embryo fibroblasts in vitro emerged. Chicken embryo fibroblasts infected with these viruses (SK770 and SK780) had a distinctive morphology, formed foci in monolayer cultures, and grew independent of anchorage in semisolid agar. Bone marrow cells were not transformed by these viruses. Another virus (SK790) with similar properties emerged during serial subcultures of chicken embryo fibroblasts after a single infection with tdB77. The 50S to 70S RNAs isolated from these viruses contained a tdB77-sized genome (7.6 kilobases), 8.7-and 5.7-kilobase RNAs, and either a 4.1-kilobase RNA or a 4.6-kilobase RNA. These RNAs did not hybridize with cDNA's representing the src, erb, mac, and myb genes of avian acute transforming viruses. Cells transformed by any one of the SK viruses (SK770, SK780, or SK790) synthesized two novel gag-related polyproteins having mol wt of 110,000 (p110) and 125,000 (p125). We investigated the compositions of these proteins with monospecific antiviral protein sera. We found that p110 was a gag-pol fusion protein which contained antigenic determinants of p19, p27, and reverse transcriptase and that p125 contained only gag determinants, leaving 49,000 daltons which was antigenically unrelated to the structural and replicative proteins of avian leukosis viruses. An analysis of the SK viral RNAs with specific DNA probes indicated that the 5.7-kilobase RNA contained gag sequences but lacked pol sequences and, therefore, probably encoded p125. The transforming ability, the deleted genome, and the induced polyproteins of the SK viruses were reminiscent of the properties of several replication-defective acute transforming viruses. (Author abstract) (51 Refs)

15. SRC GENES OF TEN ROUS SARCOMA VIRUS STRAINS, INCLUDING TWO REPORTEDLY TRANSDUCED FROM THE CELL, ARE COMPLETELY ALLELIC; PUTATIVE MARKERS OF TRANSDUCTION ARE NOT DETECTED.

Lee WH, Nunn M, Duesberg PH
Dept. Molecular Biology, Univ. California, Berkeley, CA, 94720
J Virol; 39(3):758-776 1981

We have compared the src-specific sequences of eight RSV strains and of two rRSV's to each other and to a molecular clone of the src-related chicken locus. Our comparisons are based on RNase T1-resistant oligonucleotides of RNA hybridized to src-specific cDNA, which was prepared by hybridizing RSV cDNA with RNA of isogenic src deletion mutants, or to a cloned cellular src-related DNA. All of the approx 20 src-oligonucleotides of a given RSV strain were recovered by src-specific cDNA's of all other RSV strains or by cellular src-related DNA. The number of oligonucleotides varied slightly with the length of the src deletion used to prepare src-specific cDNA, thus providing a measure for src deletion mutants. Our data indicate that the src genes are about 98% conserved and completely allelic with only scattered single nucleotide differences in certain variable regions which are subject to point mutations. We cannot distinguish between a cellular and viral origin of rRSV's. However, the following are not compatible with a cellular origin of rRSV's. (i) The only putative oligonucleotide marker which is exclusively shared by the two rRSV's studied and which differs from a parental counterpart in a single base was not detectable in cellular src-related DNA. (ii) The number of different allelic src markers observed by us and others in rRSV's was too large to derive from one or two known cellular src-related loci. (iii) The known absence of linkage of the cellular src-related locus with other virion sequences was extended to all non-src oligonucleotides, including some mapping directly adjacent to src. This is difficult to reconcile with the claim that transformation-defective, partial src deletion mutants of RSV which contain both, one, or possibly no src termini nevertheless transduce at the same frequencies, even though homologous, single or double illegitimate recombinations would be involved. Given (i) our evidence that src genes are subject to point mutation under selective conditions similar to those prevailing when rRSV's were generated and (ii) the lack of absolute evidence for the clonal purity of the transformation-defective, partial src deletion mutants of RSV used to generate rRSV's, we submit that the src genes of rRSV's could have been generated by cross-reactivation of nonoverlapping src deletions or mutation of src variants possibly present in transformation-defective, partial src deletion mutants of RSV. (52 Refs)

16. INTERACTION OF PSORALEN DERIVATIVES WITH THE RNA GENOME OF ROUS SARCOMA VIRUS.

Swanstrom R, Hallick LM, Jackson J, Hearst JE, Bishop JM
Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143
Virology; 113(2):613-622 1981

We have examined the interaction of several psoralen derivatives with the RNA genome of Rous sarcoma virus (RSV). In the presence of long-wavelength UV light, 4',5'8,-trimethylpsoralen (trioxsalen) and its 4'-hydroxymethyl (HMT) and 4'-aminomethyl (AMT) derivatives all efficiently crosslinked the subunits of the RSV 70 S RNA complex into a nondissociable state. These psoralen derivatives also were able to penetrate virions and crosslink the RNA complex in situ. The ability of psoralen to crosslink the subunits of viral RNA in situ is consistent with the presumption that the RSV genome exists as a complex within the virion and suggests an approach for a detailed examination of regions of secondary structure. The addition of psoralen adducts to viral RNA correlated with a loss of virus infectivity and a decrease in the amount and length of viral DNA synthesized in vitro and in vivo. Our results indicate that the RSV genome contains a significant amount of secondary structure that is reactive with psoralen compounds and that the addition of HMT to viral RNA causes premature chain termination during viral DNA synthesis. (Author abstract) (42 Refs)

17. ONCOGENICITY OF GIBBON RETROVIRUS DETERMINED BY LEUKEMIA-SPECIFIC GENOMIC SEQUENCES.

Sun L, Kawakami TG
(c/o Kawakami) Comparative Oncology Lab., Univ. California, Davis, CA, 95616
Virology; 114(1):261-264 1981

The genome of a Type C virus, GaLV-5, isolated from several clinically healthy gibbons, was examined for the presence of nucleotide sequences related to the myelogenous leukemia-specific 'MYE' RNA and the lymphocytic leukemia-specific 'LYM' RNA which have been isolated from several strains of

oncogenic GaLV. The genomes of these oncogenic GaLVs were each separated into sequences homologous and nonhomologous to GaLV-5. Based on hybridization analysis, the nonhomologous RNA fractions were found to contain either the MYE RNA or the LYM RNA. This indicates that the leukemia-specific RNAs are lacking in the genome of GaLV-5. This lack was further confirmed by analysis of hybridization kinetics. The presence of leukemia-specific RNA sequences in GaLV genomes that were isolated from animals with leukemia and the absence of these sequences in virus strains isolated from animals free of clinical symptoms of leukemia indicate that the leukemia-specific RNA sequences are closely associated with the oncogenesis of leukemia in gibbons. (Author abstract) (17 Refs)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

18. ACTIVATION OF RELATED TRANSFORMING GENES IN MOUSE AND HUMAN MAMMARY CARCINOMAS.

Lane MA, Sainten A, Cooper GM
Sidney Farber Cancer Inst., Boston, MA, 02115
Proc Natl Acad Sci USA; 78(8):5185-5189 1981

High mol wt DNAs of five tumors induced by mouse mammary tumor virus (MMTV), two mouse mammary tumors induced by a chemical carcinogen, and one human mammary tumor cell line (MCF-7) were assayed for the presence of transmissible activated transforming genes by transfection of NIH 3T3 mouse cells. DNAs of all five MMTV-induced tumors, one chemical carcinogen-induced tumor, and the human tumor cell line induced transformation with high efficiencies (approx 0.2 transformant per ug of DNA). NIH cells transformed by DNAs of MMTV-induced tumors did not contain exogenous MMTV DNA sequences, indicating that MMTV-induced mammary carcinomas contained activated cellular transforming genes that were not linked to viral DNA. The transforming activities of DNAs of all five MMTV-induced tumors, the chemical carcinogen-induced mouse tumor, and the human tumor cell line were inactivated by digestion with the restriction endonucleases Pvu II and Sac I, but not by BamHI, EcoRi, HindIII, Kpn I, or Xho I. These results indicate that the same or closely related transforming genes were activated in six different mouse mammary carcinomas, induced by either MMTV or a chemical carcinogen, and in a human mammary carcinoma cell line. (Author abstract) (25 Refs)

19. CELL KILLING BY AVIAN LEUKOSIS VIRUSES.

Weller SK, Temin HM
McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
J Virol; 39(3):713-721 1981

Infection of chicken cells with a cytopathic avian leukosis virus resulted in the detachment of killed cells from the culture dish. The detached, dead cells contained more unintegrated viral DNA than the attached cells. These results confirm the hypothesis that cell killing after infection with a cytopathic avian leukosis virus is associated with accumulation of large amounts of unintegrated viral DNA. No accumulation of large amounts of integrated viral DNA was found in cells infected with cytopathic avian leukosis viruses. (Author abstract) (19 Refs)

20. MICROINJECTION OF VIRUSES INTO ANIMAL EGGS AND EMBRYOS. INTEGRATION OF THE GENOME OF AVIAN SARCOMA VIRUS INTO DNA OF DROSOPHILA MELANOGASTER.

Gazarian KG, Shakhbazian AK, Neznanov NS, Smirnova SG, Kiselev FL, Tatosian AG, Spitkovskii DD
M. V. Lomonosov State Univ., Moscow, USSR
Dokl Akad Nauk SSSR; 258(5):1224-1227 1981

Injection of Rous sarcoma virus into eggs of Drosophila melanogaster produced mutation affecting the eye disk. Genome of mutant flies contained DNA sequences complementary to virus RNA. (13 Refs) (In RUS)

21. ANALYSIS OF ENDOGENOUS AVIAN RETROVIRUS DNA AND RNA: VIRAL AND CELLULAR DETERMINANTS OF RETROVIRUS GENE EXPRESSION.

Baker B, Robinson H, Varmus HE, Bishop JM
(c/o Bishop) Dept. Microbiology and Immunology, Univ. California, San Francisco, San Francisco, CA, 94143
Virology; 114(1):8-22 1981

We have characterized endogenous avian retrovirus DNAs and RNAs in uninfected white leghorn chicken embryos. The embryos we studied include representations of four phenotypic classifications of chickens (gs- chf-, gs+ chf+, gs- chf+, and V+), and contained the endogenous proviruses evl, 2, 3, 4, 5, 6, 8, 9, and 15. We confirmed the previously described phenotypes and restriction maps of evl, 3, 4, 5, 6, 8, and 15, and we confirmed the phenotype for ev9 and derived a restriction map for this locus. Evaluation of the structure of the individual proviruses and characterization of the nuclear and cytoplasmic viral RNA isolated from embryos of defined genotype yielded the following major conclusions. (i) Viral RNA could not be detected for ev4, ev5, ev8, or ev15. (ii) The amount of stable RNA produced from ev1, ev3, ev6, or ev9 varied over a range of at least 100-to 300-fold and was at least 10-to 1,000-fold lower than the amounts found in cells productively infected with exogenous avian retroviruses. (iii) A deletion in the provirus at ev3 can account for three abnormalities: the production of a viral protein that results from fusion of internal regions of the gag and pol genes, the abnormal structure of a subgenomic viral RNA, and failure of the abnormal RNA to appear in the cytoplasm. (iv) A deletion in the provirus at ev6 gives rise to at least two major anomalies: the initiation of transcription at an upstream cellular promoter, and the abnormal metabolism of an ev6 viral RNA. (v) ev9 has an ostensibly normal structure; nevertheless, the metabolism of an RNA arising from this locus is aberrant. We conclude that the expression of endogenous retrovirus genes in chickens is subject to control by both viral and cellular determinants. (Author abstract) (59 Refs)

22. IDENTIFICATION AND CLONING OF ENDOGENOUS RETROVIRAL SEQUENCES PRESENT IN HUMAN DNA.

Martin MA, Bryan T, Rasheed S, Khan AS
DNA Recombinant Res. Unit, Office Scientific Director, Natl. Inst. Allergy and Infectious Diseases, NIH, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 78(8):4892-4896 1981

Using nonstringent annealing conditions and a 2.75-kilobase segment of cloned African green monkey DNA that specifically hybridizes to the proviruses of AKR ecotropic murine leukemia virus (MuLV) and baboon endogenous virus (BaEV) as a probe, we detected related sequences in three different preparations of human brain DNA fragments. The blot-hybridization pattern obtained with cleaved human DNA was similar to

that previously reported for the interaction of MuLV cDNA and cleaved mouse DNA and suggested the presence of numerous copies of retrovirus-related sequences in the human genome. The labeled 2.75-kilobase fragment derived from cloned monkey DNA was used to screen a human DNA library in Charon 4A. One clone obtained hybridized to three contiguous MuLV- and BaEV-reactive fragments of the cloned monkey DNA and to multiple fragments of human DNA including a prominent 1.0-kilobase EcoRI fragment also present in the clone. (Author abstract) (37 Refs)

23. DETECTION AND CLONING OF MURINE LEUKEMIA VIRUS-RELATED SEQUENCES FROM AFRICAN GREEN MONKEY LIVER DNA.

Martin MA, Bryan T, McCutchan TF, Chan HW
Lab. Molecular Microbiology, Natl. Inst. Allergy and Infectious Diseases, Bethesda, MD, 20205
J Virol; 39(3):835-844 1981

By using low-stringency nucleic acid hybridization conditions and specific subgenomic segments of the AKR ecotropic provirus as probes, murine leukemia virus (MuLV)-related sequences were detected in African green monkey (AGM) liver DNA. The MuLV-reactive segments present in restricted AGM DNA ranged from 1.9 kilobases (kb) to greater than 10 kb in size. On the basis of this finding, a 17-kb segment was cloned from a partial EcoRI AGM library in lambda Charon 4A which shared nearly 5 kb of homology with AKR ecotropic MuLV DNA. The MuLV-related sequences detected in restricted preparations of AGM DNA or present in the cloned monkey DNA reacted with probes mapping 2.0 to 7.0 kb from the 5' terminus of the AKR ecotropic provirus. The AGM clone also contained repeated sequences that flanked the MuLV-related segment. Labeled, subgenomic, MuLV-reactive segments of the monkey clone hybridized to multiple restriction fragments of AGM liver DNA, indicating the presence of several copies of the MuLV-related sequences. (Author abstract) (48 Refs)

24. RESTRICTION ENDONUCLEASE ANALYSIS OF MITOCHONDRIAL DNA FROM VIRUS-TRANSFORMED, TUMOR AND CONTROL CELLS OF HUMAN, HAMSTER AND AVIAN ORIGIN. SEQUENCE CONSERVATION AND INTRASPECIFIC VARIATION.

Nass MM
Dept. Therapeutic Res., Univ. Pennsylvania Sch. Medicine, Philadelphia, PA, 19104
Biochim Biophys Acta; 655(2):210-220 1981

This study compares over 70 recognition sites for restriction endonucleases on mtDNAs from various control versus malignant cells, derived from Syrian hamster, chick embryo, viper and human cells, exhibiting a wide spectrum of cellular transformation and tumor histories. Agents for transformation in vitro and in vivo include Rous sarcoma viruses, simian virus 40, polyoma virus and adenovirus. The results show a striking intraspecific sequence homogeneity of different mtDNAs regardless of tissue origin and oncogenic history. mtDNA from human biopsy specimens of tumor versus pathologically normal areas yielded indistinguishable restriction cleavage patterns reflecting either the 'wild-type' form (with 7 restriction endonucleases) or, in one individual, a variant pattern detected with HpaI. The precise position of the HpaI variant site was determined on the physical map of human mtDNA. Additional cleavage sites in the previously reported restriction map of Syrian hamster mtDNA are also presented. It is concluded that (1) mtDNA sequences in higher animal cells are highly conserved in malignant transformation; (2) no evidence for integration of viral sequences in mtDNA is apparent; (3) variant patterns in mtDNA are likely to be intraspecific polymorphisms that pre-exist neoplastic transformation. The possibility is discussed that altered regulatory interaction with the mitochondrial genome, rather than evident changes in mtDNA primary structure, determine anomalous mitochondrial functions in malignant transformation. (Author abstract) (62 Refs)

25. LACK OF AKR ECOTROPIC PROVIRUS AMPLIFICATION IN AKR LEUKEMIC THYMUSES.

Yoshimura FK, Breda M
Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
J Virol; 39(3):808-815 1981

A DNA fragment from the 3' region of a molecularly cloned AKR ecotropic provirus was identified to be specific for the AKR ecotropic murine leukemia virus (MuLV). This selected DNA fragment was used to analyze the integrated MuLV proviruses in normal and leukemic tissue DNAs of AKR mice. In comparison with a DNA fragment from the 5' region of the cloned AKR genome or one representing the entire genome, this selected probe hybridized to only a few MuLV proviruses. By comparing transformed and nontransformed tissue DNAs, it appeared that no amplification of proviral sequences related to the AKR ecotropic MuLV had occurred in thymomas of AKR mice during the development of leukemia in these animals. Analysis of the AKR ecotropic MuLV proviruses revealed a significant degree of polymorphism for these sequences among individuals in the AKR/J strain of mouse. (Author abstract) (24 Refs)

26. CLONING OF THE REGION OF MURINE MOLONEY SARCOMA VIRUS GENOME CONTAINING ONCOGEN OF THIS VIRUS.

Chumakov IM, Zabarovskii ER, Mett VL, Prasolov VS, Kiselev LL
Inst. Molecular Biology, Moscow, USSR
Dokl Akad Nauk SSSR; 259(1):219-222 1981

The results of cloning of the region of murine Moloney sarcoma virus (MSV) genome containing MSV oncogen are reported. The method is based on in vitro DNA recombination with subsequent cloning and replication. (8 Refs) (In RUS)

ANTIVIRALS

27. EFFECTS OF OUABAIN AND INTERFERON ON THE PRODUCTION OF MURINE LEUKEMIA AND VESICULAR STOMATITIS VIRUSES IN OUABAIN-SENSITIVE AND RESISTANT CELLS.

Tomita Y, Niimura Y, Kuwata T
Dept. Microbiology Sch. Medicine, Chiba Univ, Chiba, Chiba 280, Japan
Microbiol Immunol; 25(7):741-744 1981

The isolation of an ouabain (OB)-resistant JLS-V9 mouse cell line (JLS-V9OR) is described and the effects of OB and interferon (IF) on murine leukemia virus (MuLV) production in these cells were studied. The JLS-V9 cells were chronically infected with Rauscher MuLV. The JLS-V9OR cells, which grew well in the presence of 1 x 10(-3) M OB showed almost the same growth rate as the parent JLS-V9 cells regardless of the presence of OB, while the growth of JLS-V9 cells was sig-

nificantly reduced in the presence OB. The resistance of JLS-V9OR cells was not lost during cultivation for over 2 mo in the absence of OB. Results of studies on the mechanism of OB resistance indicated that the resistance of cell multiplication to OB is mainly due to the decreased sensitivity of Na+, K+-dependent ATPase to OB. OB suppressed MuLV production in OB-sensitive JLS-V9 cells. However, in JLS-V9OR cells, OB had no significant effect on MuLV production. These results indicated a close correlation between resistance of cells to OB in cell growth and MuLV production. OB blocked the antiviral activity of IF against vesicular stomatitis virus (VSV) when OB-sensitive JLS-V9 cells were simultaneously treated with 100 Units/ml of mouse IF and 5 x 10(-4) M OB. However in OB-resistant cells, 100 Units/ml of IF suppressed the growth of VSV to below 1% of that of the control in the presence of 5 x 10(-4) M OB as well as in its absence. Thus, in JLS-V9 cells, OB suppressed the antiviral action of IF, whereas this effect of OB was not observed in the JLS-V9OR cells. (16 Refs)

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VIRAL PROTEINS

1. SEQUENCE-SPECIFIC ANTIBODIES SHOW THAT MATURATION OF MOLONEY LEUKEMIA VIRUS ENVELOPE POLYPROTEIN INVOLVES REMOVAL OF A COOH-TERMINAL PEPTIDE.

Green N, Shinnick TM, Witte O, Ponticelli A, Sutcliffe JG, Lerner RA
Committee Study Molecular Genetics, Res. Inst. Scripps Clinic, La Jolla, CA
Proc Natl Acad Sci USA; 78(10):6023-6027 1981

We followed maturation of the glycosylated envelope polyprotein Pr80env of a murine retrovirus by using antisera specific to subregions of the protein, including an antiserum directed against a synthetic peptide corresponding to the COOH-terminus of Pr80env. Shortly after synthesis and glycosylation, Pr80env is cleaved into two species, gp70 and Pr15E, that are found associated, perhaps through disulfide bonds, in infected cells. Pr15E is further cleaved at the time of virus maturation to form virus pr otein p15E. NH2-Terminal protein sequence analysis showed that Pr15E had an NH2 terminus in common with p15E. Pr15E, but not p15E, is precipitated by antibody against the COOH-terminal peptide; hence, p15E is missing a

HE 20.3172/2

peptide at the COOH-terminus. Our data indicate that Pr15E is the predominant species in cells and p15E is the major species in virus. (Author abstract) (32 Refs)

2. IMMUNOCHEMICAL REACTIVITY OF SIMIAN SARCOMA VIRUS POLYPEPTIDES ISOLATED BY PREPARATIVE SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS.

Johnson JC, Higuchi T, Geissler E
Dept. Biological Sciences, Old Dominion Univ., Norfolk, VA, 23508
Can J Microbiol; 27(2):238-242 1981

The polypeptides associated with a zonal centrifugation purified simian sarcoma virus propagated in lymphoblastoid NC-37 cells were isolated by preparative polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) using a procedure designed to minimize the loss of immunochemical reactivity. The proteins p10, p15, p28, p36, p44, p75, and p86 were obtained in large yield and high degree of homogeneity. The electrophoretically purified p28 was analyzed by competition radioimmunoassay using antiserum to a pore exclusion and ion exchange purified simian sarcoma virus p28. Complete competition was observed with extracts of simian sarcoma virus infected cells. No competition was observed with uninfected or unrelated, infected cell extracts. The antigen-antibody affinity as measured by the slope of the competition curve using antiserum to p28 and 125I-labeled and electrophoretically purified p28 was the same as that for the p28 released from sonication-disrupted simian sarcoma virus. The data indicates that preparative purifications by polyacrylamide gel electrophoresis in the presence of SDS may be generally applicable for the isolation of proteins with essentially the same immunospecificities and affinity for a specific antiserum as proteins isolated by procedures that avoid the use of SDS and electrophoresis. (Author abstract) (21 Refs)

3. CLEAVAGE OF FOUR AVIAN SARCOMA VIRUS POLYPROTEINS WITH VIRION PROTEASE P15 REMOVES GAG SEQUENCES AND YIELDS LARGE FRAGMENTS THAT FUNCTION AS TYROSINE PHOSPHOACCEPTORS IN VITRO.

Ghysdael J, Neil JC, Vogt PK
Dept. Microbiology, Univ. Southern California Sch. Medicine, 2011 Zonal Ave., Los Angeles, CA, 90033
Proc Natl Acad Sci USA; 78(9,part2):5847-5851 1981

The transformation-specific polyproteins of avian sarcoma viruses PRCII, PRCII-p, Fujinami sarcoma virus (FSV), and Esh sarcoma virus (ESV) consist of two domains, one derived from a partial viral gag gene and the other representing an apparently cell-derived insert in the defective viral genome. These gag-linked proteins were cleaved with retrovirion protease p15. Cleavage of PRCII-p polyprotein P170 , P105 of PRCII, and P140 of FSV occurred within the gag domain and generated fragments of Mr 130,000, 70,000, and 115,000, respectively, containing all of the transformation-specific sequences linked to a remnant of the original gag sequences. ESV P80 was cleaved inside the transformation-specific domain, yielding a Mr 35,000-38,000 fragment from the NH2-terminal half of the molecule consisting of the entire gag portion and some non-gag sequences and a Mr 48,000 fragment containing most of the transformation-specific sequences. The tyrosine phosphorylation sites of the polyproteins were found in every case in the transformation-specific fragments. The major serine phosphorylation site of ESV P80 was found to reside in the Mr 35,000-38,000 gag-containing fragment, probably within the transformation-specific sequences of that cleavage product. Removal of all of the gag domain of ESV P80 or most of the gag domain in PRCII-p P170, PRCII P105, and FSV P140 does not affect their ability to be phosphorylated by the polyprotein-associated tyrosine-specific protein kinase activities. This observation suggests that the gag sequences of the polyproteins of classes II (PRCII-p, PRCII, and FSV) and III (ESV) avian sarcoma viruses may not be required for this enzymatic function, which appears to be of importance in transformation. (Author abstract) (31 Refs)

4. PROTEOLYTIC PROCESSING OF AVIAN AND SIMIAN SARCOMA AND LEUKEMIA VIRAL PROTEINS.

Konze-Thomas B, Von der Helm K
Max v. Pettenkofer-Institut fur Hygiene und Med. Mikrobiolgie, Ludwig-Maximilians-Universitat, Pettenkoferstrasse 91, D-8000 Munich 2, W. Germany
Haematol Blood Transfus; 26:409-413 1981

The cleavage of a 110,000 dalton fusion protein of the replication-defective avian acute leukemia virus MC 29 and a gag protein precursor pr65 of Simian sarcoma virus by p15 into discrete smaller proteins is described. A discussion is also presented on how p15 could be used as a tool for mapping transformation proteins. (15 Refs)

5. PRCII, A REPRESENTATIVE OF A NEW CLASS OF AVIAN SARCOMA VIRUSES.

Vogt PK, Neil JC, Moscovici C, Breitman ML
Dept. Microbiology, Univ. Southern California Medical Center, 2025 Zonal Ave., Los Angeles, CA, 20014
Haematol Blood Transfus; 26:424-428 1981

Preliminary studies of Poultry Research Center Virus II (PRCII), a replication-defective avian sarcoma virus, showed that the virus lacks the src transforming gene but codes for a transformation-specific gag-related polyprotein (p105). Tryptic peptides maps of p105 did not coincide with those of the transformation-specific proteins of Rous sarcoma virus and avian acute leukemia viruses. (34 Refs)

6. THE TRANSFORMATION-SPECIFIC PROTEIN PP60SRC FROM AN AVIAN SARCOMA VIRUS.

Moelling K, Owada MK, Donner P, Bunte T
Max-Planck-Institut fur Molekular Genetik, Ihnestrasse 63-73, 1000 Berlin 33, W. Germany
Haematol Blood Transfus; 26:405-408 1981

The isolation, purification, and characterization of Schmidt-Ruppin virus, subgroup D, transformation-specific protein pp60src are reported. The protein was located in the virion membrane but was not accessible to antibodies, surface iodination, or protease. (7 Refs)

7. CHARACTERIZATION OF SITES FOR TYROSINE PHOSPHORYLATION IN THE TRANSFORMING PROTEIN OF ROUS SARCOMA VIRUS (PP60V-SRC) AND ITS NORMAL CELLULAR HOMOLOGUE (PP60C-SRC).

Smart JE, Oppermann H, Czernilofsky AP, Purchio AF, Erikson RL, Bishop JM
Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724
Proc Natl Acad Sci USA; 78(10):6013-6017 1981

The transforming protein of Rous sarcoma virus (pp60v-src) and its normal cellular homologue (pp60c-src) appear to be protein kinases that phosphorylate tyrosine in a variety of protein substrates. In addition, pp60v-src and pp60c-src are them-

selves phosphorylated on serine and tyrosine. It is likely that these phosphorylations serve to regulate the function(s) of pp60v-src and pp60c-src. We have therefore characterized the sites of tyrosine phosphorylation in the two proteins. Tyrosine phosphorylation of pp60v-src in infected cells occurs mainly (if not entirely) at residue 419 in the deduced amino acid sequence of the protein. Surrounding this residue is the sequence Leu-Ile-Glu-Asp-Asn-Glu-Tyr(P)-Thr-Ala-Arg. This peptide is distinguished by the fact that three out of the four amino acids that precede the phosphorylated tyrosine are acidic in nature. These results define what may prove to be a widely used site for tyrosine phosphorylation in the regulation of cellular function. The same site was phosphorylated when partially purified pp60v-src was used in a phosphotransfer reaction in vitro. The results with pp60c-src were more complex. the site of tyrosine phosphorylation in vitro appeared to be the same as that found in pp60v-src. By contrast, phosphorylation of pp60c-src in vivo apparently occurred at a different, and currently unidentified, tyrosine residue. It is therefore possible that pp60v-src and pp60c-src respond differently to regulatory influences in the intact cell. (Author abstract) (34 Refs)

8. GENERATION OF BALB-MUSV AND HA-MUSV BY TYPE C VIRUS TRANSDUCTION OF HOMOLOGOUS TRANSFORMING GENES FROM DIFFERENT SPECIES.

Andersen PR, Devare SG, Tronick SR, Ellis RW, Aaronson SA, Scolnick EM
Lab. Cellular and Molecular Biology, NCI, Bethesda, MD, 20205
Cell; 26(1):129-134 1981

The nature of the cell-derived (bas) sequences of BALB-MuSV, a spontaneous mouse sarcoma virus isolate, was determined. Molecularly cloned bas sequences demonstrated no detectable homology with the onc genes of other mouse transforming viruses, but exhibited a high degree of sequence homology with the ras gene of the rat-derived Harvey murine sarcoma virus (Ha-MuSV) genome. The Ha-MuSV cell-derived sequence (ras) shared a colinear 750 bp region of homology with bas. Moreover, BALB-MuSV transformation was associated with the expression of high levels of a 21,000 dalton protein, immunologically related to the ras gene product, p21. Thus bas and ras represent retroviral transforming gene homologs that were independently transduced by mouse Type C viruses from the genomes of different species. (Author abstract) (41 Refs)

9. A CELLULAR PROTEIN PHOSPHORYLATED BY THE AVIAN SARCOMA VIRUS TRANSFORMING GENE PRODUCT.

Erikson E, Erikson RL
Health Sciences Center, Univ. Colorado, 4200 E. Ninth Ave., Denver, CO, 80262
Haematol Blood Transfus; 26:397-401 1981

Experiments are described which demonstrate that a newly phosphorylated protein in avian sarcoma virus (ASV)-transformed chicken cells with a mol wt of approx 34,000 (34K) and an isoelectric focusing of about 7.5 is a src-specific substrate found in normal cells and that upon transformation it is phosphorylated in ASV-infected cells of both avian and mammalian origin. This protein appears to be a substrate for the phosphotransferase activity associated with pp60src. This protein, or an analogous protein was partially phosphorylated in all ASV-transformed cells examined to date. The pp60src-specific nature of the phosphorylation is shown by the phosphorylation of 34K by pp60src in vitro at a site identical to that phosphorylated in vitro at a site identical to that phosphorylated in transformed cells. This result strongly suggests that 34K is a direct rather than indirect substrate of pp60src in the cell. This result also suggests that pp60src acts as a protein kinase in the transformed cell as well as in vitro and that it mediates transformation, at least in part, by phosphorylation of 34K and perhaps of other normal cell proteins. This conclusion does not eliminate the possibility that pp60src may have other functions that influence transformation. (11 Refs)

10. CORRELATED LOSS OF THE TRANSFORMED PHENOTYPE AND PP60SRC-ASSOCIATED PROTEIN KINASE ACTIVITY.

Friis RR, Ziemiecki A, Bosch V, Boschek CB, Bauer H
Institut fur Virologie, Justus-Liebig-Universitat, Frankfurter Strasse 107, 6300 Giessen, W. Germany
Haematol Blood Transfus; 26:402-404 1981

NY68 cells were infected with a transformation-defective, temperature-sensitive mutant of Rous sarcoma virus. The protein kinase activity of the viral gene product pp60src declined rapidly when the infected cells were shifted to 42 C (nonpermissive for virus growth). Loss of kinase activity correlated with the reversion of the transformed cells to a normal phenotype (evidenced by changes in cell microfilaments). New protein synthesis was not required for the reversion. (9 Refs)

VIRAL RNA

11. NUCLEOTIDE SEQUENCE OF MOLONEY MURINE LEUKAEMIA VIRUS.

Shinnick TM, Lerner RA, Sutcliffe JG
Committee Study Molecular Genetics, Res. Inst. Scripps Clinic, La Jolla, CA, 92037
Nature; 293(5833):543-548 1981

We have determined the 8,332-nucleotide structure of the genome of Moloney murine leukemia virus. The coding frame for the gag gene is the same as that for pol, separated only by a single amber triplet. The pol gene encodes 132,000 daltons of protein, larger than the 70,000 daltons required for the reverse transcriptase. The env gene overlaps the pol gene. (Author abstract) (99 Refs)

12. COMPLETE NUCLEOTIDE SEQUENCE AND ORGANIZATION OF THE MOLONEY MURINE SARCOMA VIRUS GENOME.

Reddy EP, Smith MJ, Aaronson SA
Lab. Cellular and Molecular Biology, NCI, Bethesda, MD, 20205
Science; 214(4519):445-450 1981

The complete nucleotide sequence of a mammalian transforming retrovirus, Moloney murine sarcoma virus, has been determined. MSV, a recombinant virus derived of helper viral and cellular sequences, possesses termini resembling prokaryotic transposable elements. The viral genome has the coding capacity for the Moloney murine leukemia virus gag gene product and contains large deletions in pol and env genes. A large open reading frame encompassing its cell-derived sequences codes for its putative transforming protein. The nature of some of the important domains in the viral genome has been established, and their structure is discussed in relation to their function. (Author abstract) (48 Refs)

13. UNTRANSLATED SEQUENCES OF GENOMIC AND INTRACELLULAR ROUS SARCOMA VIRUS RNAS (MEETING ABSTRACT).

Bromley PA, Darlix JL, Spahr PF
Departement de Biologie Moleculaire, Universite de Geneve, 30, quai Ernest-Ansermet, CH-1211 Geneve 4, Switzerland
Experientia; 37(6):640 1981

We have determined the nucleotide sequence of the untranslated region preceding the 5'-end proximal gene ('gag') of Rous Sarcoma Virus (RSV) 35S genomic RNA. The relatively long leader sequence (370 nucleotides) possesses unique features that suggest a novel mechanism for the initiation of the 'gag' gene translation. S1 nuclease mapping using DNA fragments complementary to the leader of RSV genomic RNA indicate that this sequence is conserved in the crude 35S intracellular RSV polysomal RNA. The purification of the various intracellular RSV specific RNA using affinity chromatography and the characterization of the 35S species will be described. The complete nucleotide sequence of the junction between the gene 'gag' and 'pol' has also been determined and its implication for the existence of a separate mRNA for the 'pol' gene will be discussed. An analysis by UV irradiation of RSV indicates that the intercistronic region 'gag'-'pol' interacts with the virion protein p 19. (no Refs)

14. TWO VIRUS-SPECIFIC RNA SPECIES ARE PRESENT IN CELLS TRANSFORMED BY DEFECTIVE LEUKEMIA VIRUS 0K10.

Chiswell DJ, Ramsay G, Hayman MJ
Imperial Cancer Res. Fund, Lincoln's Inn Fields, London, WC2A 3PX, England
J Virol; 40(1):301-304 1981

OK10 is a defective leukemia virus which shares some biological and biochemical properties of avian myelocytomatosis virus (MC29). We investigated the pattern of transcription of OK10 in both quail and chicken cells. In both cell types, OK10 produced two polyadenylated RNA species of 8.6 and 3.5 kilobases, which both contained sequences derived from the 5' end of the genome and also the presumed transforming gene (myc). This is a novel form of expression for defective leukemia viruses of the MC29 subgroup and may indicate that there is an as-yet-unidentified protein produced in OK10-infected cells which may be involved in transformation. (Author abstract) (25 Refs)

15. IDENTIFICATION OF THE AVIAN MYELOBLASTOSIS VIRUS GENOME.

Souza LM, Bergmann DG, Baluda MA
(c/o Baluda) Dept. Microbiology and Immunology, UCLA Cancer Immunology Center, 921 Westwood Blvd., Los Angeles, CA, 90024
Haematol Blood Transfus; 26:429-431 1981

Unintegrated and integrated viral DNA intermediates were characterized; results are summarized. Linear viral DNA isolated from cytoplasm of helper virus (MAV-1 or MAV-2) infected chicken embryonic fibroblasts was distinguished by Hind III restriction endonuclease cleavage. The arrangement of endogenous proviral DNA in the chicken genome was determined prior to avian myeloblastosis virus (AMV) infection. Infections were carried out in vitro and in vivo using AMV-S, AMV-B, and AMV-C stocks. Characterization by restriction enzyme fragmentation, construction of recombinant DNA clones, and hybridization studies is described. Restriction enzyme maps of the presumptive AMV and MAV-1-like genomes are presented. Hybridization studies demonstrated that the AMV genome contains a cellular substitution; physical mapping places this substitution in the region normally occupied by the envelope gene. The substitution may be responsible for leukemogenic potential and defectiveness. Differences between AMV and avian defective transforming viruses are listed. (7 Refs)

16. GENETIC STRUCTURE, TRANSFORMING SEQUENCE, AND GENE PRODUCT OF AVIAN SARCOMA VIRUS UR1.

Wang L-H, Feldman R, Shibuya M, Hanafusa H, Notter MF, Balduzzi PC
Rockefeller Univ., New York, NY, 10021
J Virol; 40(1):258-267 1981

We analyzed the genetic structure and gene products of the newly isolated avian sarcoma virus UR1, which recently has been shown to be replication defective and to contain no sequences homologous to the src gene of Rous sarcoma virus. The sizes of the genomic RNAs of UR1 and its associated helper virus, UR1AV, were determined to be 29S and 35S (5.9 and 8.5 kilobases), respectively, by gel electrophoresis and sucrose gradient sedimentation. RNase T1 oligonucleotide mapping of purified viral RNAs indicated that UR1 RNA contains eight unique oligonucleotides in the middle of the genome and shares four 5'-terminal and three 3'-terminal oligonucleotides with UR1AV RNA. The unique sequences of UR1 and Fujinami sarcoma virus were found to be closely related to each other by molecular hybridization of UR1 RNA with DNA complementary to the unique sequence of Fujinami sarcoma virus RNA, but minor differences were found by oligonucleotide fingerprinting. In the regions flanking the unique sequences, UR1 and Fujinami sarcoma viral RNAs contain distinct oligonucleotides, which are shared with oligonucleotides of the respective helper viral RNAs. Cells transformed with UR1 produce a single 29S RNA species which contains a UR1 unique sequence; this species is most likely the mRNA coding for the transforming protein. In UR1-transformed cells, a phosphoprotein of 150,000 daltons (p150) was detected by immunoprecipitation with antiserum against gag proteins. p150 was associated with a protein kinase activity that was capable of phosphorylating p150 itself, Ig G of antiserum, and a soluble substrate, alpha-casein. This enzyme transferred phosphate exclusively to tyrosine residues of substrates in vitro, but p150 labeled in vivo with 32P contained both phosphoserine and phosphotyrosine. The in vitro kinase reaction was not affected by the presence of cyclic AMP or cyclic GMP and strongly preferred Mn++ over Mg++. Thus, the properties of UR1 protein are almost identical to those of Fujinami sarcoma virus protein. (Author abstract) (26 Refs)

17. IN VITRO TRANSLATION OF AVIAN MYELOBLASTOSIS VIRUS RNA.

Anderson SM, Chen JH
Tumor Virus Genetics Lab., NIH, Bethesda, MD, 20205
J Virol; 40(1):107-117 1981

Avian myeloblastosis virus (AMV)-infected cells contain two viral mRNA's, a genome-sized 34S (7.5-kilobase) mRNA and a 21S (2.5-kilobase) subgenomic mRNA, which contains the AMV-specific sequences (myb sequences). We found that AMV virions packaged both the 7.5-kilobase full-length genomic RNA and the 2.5-kilobase subgenomic RNA. In vitro translation of AMV virion RNA sized by sucrose density gradient centrifugation yielded 76,000-, 56,000-, 48,500-, 47,000-, and 32,000-dalton products. The 76,000-dalton protein was coded for by RNA throughout the gradient, but the peak of

activity was at 34S to 35S. The 56,000-, 48,500-, and 32,000-dalton proteins were encoded in a 21S RNA, and the 47,000-dalton protein was encoded in an RNA of approx 24S. The 76,000-dalton protein was identified as Pr76gag. based upon immunoprecipitation with specific antiserum and the presence of the 19* dipeptide. 7-Methylguanosine triphosphate inhibited the syntheses of Pr76gag and the 56,000-, 48,500-, and 32,000-dalton proteins, but not the synthesis of the 47,000-dalton protein. the 56,000-, 48,500-, 47,000-, and 32,000-dalton proteins were not immunoprecipitated by anti-gag, anti-reverse transcriptase, or anti-gp85 antiserum. Two-dimensional peptide maps of the 56,000-and 48,500-dalton proteins indicated that they were unique. In vitro translational products of myeloblastosis-associated virus 1 were also analyzed to aid in the identification of the AMV myb gene product(s); the translational products analyzed included Pr76gag, p60env, and a 56,000-dalton polypeptide which apparently was not identical to the 56,000-dalton AMV translational product, as determined by two-dimensional peptide mapping. Our data indicated that one of these proteins (56,000, 48,500, or 32,000 daltons) may represent the product of the AMV myb gene and, therefore, the putative transforming protein(s) of AMV. (Author abstract) (57 Refs)

18. GENETICS OF LEUKEMOGENESIS BY AVIAN LEUKOSIS VIRUSES.

Coffin JM, Tsichlis PN, Robinson HL
Dept. Molecular Biology, Tufts Univ., 136 Harrison Ave., Boston, MA, 02111
Haematol Blood Transfus; 26:432-438 1981

Biological differences between endogenous and exogenous viruses of chickens were explored in order to obtain information regarding the roles of various portions of the exogenous virus genome in pathogenicity, particularly in lymphoid leukosis. When genomes of various endogenous and exogenous viruses were subjected to T1 oligonucleotide mapping, it was concluded that these viruses represent two distinct but closely related lineages. The greatest diversity is in the U3 region near the 3' end of the genome. Studies were performed to determine the roles of various regions in regard to growth and pathogenicity of the viruses, using a series of recombinants between RAV-0 and either Pr-B or its td derivative. Repeated selection of the U3cx allele suggested responsibility for difference in growth rate between endogenous and exogenous viruses. Further studies with either endogenous or recombinant viruses indicated that the U3cx region is the major determinant responsible for the more rapid growth of exogenous viruses. A discussion is presented of the role of the U3 region in leukemogenesis, which clearly indicates the separation of growth rate from leukemogenicity. It is suggested that the nonpathogenicity of endogenous viruses is an adaptation to their quiescent lifestyle, which involves lack of harm to the host. The experiments described should be useful in identifying and characterizing the region of the virus genomes relevant to this adaptation and exploiting differences in various viruses in order to probe molecular mechanisms of viral leukemogenesis. (27 Refs)

19. IDENTIFICATION OF A RNA POLYMERASE II INITIATION SITE IN THE LONG TERMINAL REPEA T OF MOLONEY MURINE LEUKEMIA VIRAL DNA.

Fuhrman SA, Van Beveren C, Verma IM
Dept. Biology, Univ. California at San Diego, La Jolla, CA, 92093
Proc Natl Acad Sci USA; 78(9,part2):5411-5415 1981

We have used a soluble in vitro RNA polymerase II transcription system to define the site of initiation of Moloney murine leukemia viral RNA synthesis. Molecularly cloned integrated and unintegrated Moloney murine leukemia virus DNAs were used as templates. The 5' ends of in vitro transcripts and virion RNA of Moloney murine leukemia virus were compared by nuclease S1 protection experiments. Our results indicate that viral sequences upstream of the in vivo cap site are implicated in the transcription of viral RNA and that the 5' end of an in vitro transcript derived from an integrated Moloney murine leukemia virus clone corresponds to the 5' end of viral genomic RNA. (Author abstract) (41 Refs)

20. QUANTITATION OF MURINE MAMMARY TUMOR VIRUS-RELATED RNA IN MAMMARY TISSUES OF LOW- AND HIGH-MAMMARY-TUMOR-INCIDENCE MOUSE STRAINS.

Marcus SL, Smith SW, Sarkar NH
Lab. Molecular Virology, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
J Virol; 40(1):87-95 1981

Lactating mammary glands and hormonally induced mammary tumors of BALB/c mice from three geographically separated breeding colonies were examined by molecular hybridization, using murine mammary tumor virus (MuMTV)cDNA representing the entire viral genome to determine the amount of MuMTV-related RNA expressed in these tissues. The RNA extracted from these tissues by the classical sodium dodecyl sulfate-pronase, phenol-chloroform procedure (method 1) contained barely detectable levels of MuMTV-related sequences. In contrast, both normal lactating mammary glands and hormonally induced mammary tumors of these mice were found to contain approx one to two copies of the MuMTV genome per cell by using a new procedure in which the RNA was extracted with guanidine derivatives (method 2). No significant differences in the MuMTV-related RNA content of the BALB/c mammary tissues were observed regardless of their colony of origin. Our results suggest that expression of MuMTV RNA does not change in BALB/c mammary glands during transformation to a malignant state and that MuMTV expression does not play a role in tumorigenesis in these mice. In view of the increased recovery of MuMTV-related RNA from BALB/c mice with method 2, we compared the level of MuMTV RNA expression in lactating mammary glands and mammary tumors of other mouse strains, including C57BL/6 and RIII, using both extraction methods. Yields of MuMTV-related RNA from mammary tissues increased by as much as 35-to 40-fold, using method 2 as compared with method 1. Therefore method 2, involving guanidine derivatives, appears to be the method of choice for MuMTV-related RNA extraction from the mammary tissues o f certain strains of mice, particularly those expressing relatively low levels of MuMTV RNA. (Author abstract) (20 Refs)

21. VIRAL GENE EXPRESSION CELLS TRANSFORMED BY SIMIAN SARCOMA VIRUS, AND INFECTIOUS PRIMATE TYPE C RETROVIRUS.

Bergholz CM
No affiliation given
Haematol Blood Transfus; 26:524-526 1981

Studies on the viral gene expression in Simian sarcoma virus (SSV)-transformed virus producing marmoset monkey cells [HF/SSV(SSAV)] and SSV-transformed nonproducer marmoset cell lines (HF/SSV-NP) are described. Using antiserum specific for SSV(SSAV: nontransforming associated virus) p30 and another specific for SSAV envelope proteins, it was dem-

onstrated that anti-SSAV p30 precipitates predominantly polypeptides of approx 30,000 and 60,000-62,000 daltons and, in addition, polypeptides of approx 72,000 and 52,000 daltons. Results of pulse-chase experiments indicated that the 60,000 dalton protein (pr60gag) is processed to yield p30, the major viral core protein. Using antiserum specific for gp70 and p15E/p12E, large amounts of 82,000 dalton polypeptide were precipitated; results of pulse-chase experiments indicated that it is an envelope precursor protein processed to yield p15E and p12E. Two virus-specific phosphoproteins, the pr60gag and p12 were revealed by electrophoresis. No phosphorylated proteins were precipitated by antiserum specific for SSAV envelope proteins. IgG was phosphorylated in extracts of both HF/SSV(SSAV) and HF/SSAV cells, but not in extracts of HF/SSV-NP cells. Expression of SSAV-related sequences by the SSV provirus was investigated by reacting an SSAV circular DNA with increasing concentrations of RNA extracted from two HF/SSV-NP cell lines. The results indicated that SSAV-related sequences were transcribed but represented a smaller proportion of the total cell RNA relative to virus producing cells. Comparison of max levels of hybridization indicated that less of the SSAV genome is represented in the SSV transcripts relative to RNA in SSAV-infected cells. The failure to detect virus-specific proteins in five HF/SSV-NP cells lines shown to contain SSAV-related cytoplasmic RNA suggested that translation of this RNA may be restricted. (1 Ref)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

22. INTRAMOLECULAR INTEGRATION WITHIN MOLONEY MURINE LEUKEMIA VIRUS DNA.

Shoemaker C, Hoffmann J, Goff SP, Baltimore D
Center Cancer Res., Dept. Biology, Massachusetts Inst. Technology, Cambridge, MA, 02139
J Virol; 40(1):164-172 1981

By screening a library of unintegrated, circular Moloney murine leukemia virus (M-MuLV) DNA cloned in lambda phage, we found that approx 20% of the M-MuLV DNA inserts contained internal sequence deletions or inversions. Restriction enzyme mapping demonstrated that the deleted segments frequently abutted a long terminal repeat (LTR) sequence, whereas the inverted segments were usually flanked by LTR sequences, suggesting that many of the variants arose as a consequence of M-MuLV DNA molecules integrating within their own DNA. Nucleotide sequencing also suggested that most of the variant inserts were generated by autointegration. One of the recombinant M-MuLV DNA inserts contained a large inverted repeat of a unique M-MuLV sequence abutting an LTR. This molecule was shown by nucleotide sequencing to have arisen by an M-MuLV DNA molecule integrating within a second M-MuLV DNA molecule before cloning. The autointegrated M-MuLV DNA had generally lost two base pairs from the LTR sequence at each junction with target site DNA, whereas a four-base-pair direct repeat of target site DNA flanked the integrated viral DNA. Nucleotide sequencing of preintegration target site DNA showed that this four-base-pair direct repeat was present only once before integration and was thus reiterated by the integration event. The results obtained from the autointegrated clones were supported by nucleotide sequencing of the host-virus junction of two cloned M-MuLV integrated proviruses obtained from infected rat cells. Detailed analysis of the different unique target site sequences revealed no obvious common features. (Author abstract) (33 Refs)

23. FV-1 HOST RESTRICTION OF FRIEND LEUKEMIA VIRUS: ANALYSIS OF UNINTEGRATED PROVIRAL DNA.

Chinsky J, Soeiro R
Dept. Cell Biology, Albert Einstein Coll. Medicine, Bronx, NY, 10461
J Virol; 40(1):45-55 1981

The murine gene Fv-1 predominantly controls the outcome of infection by murine ecotropic retroviruses. The inhibition of virus replication by the Fv-1 gene product has been determined to be at an early stage in virus replication. Mechanistically, its effect appears to be on the accumulation of unintegrated proviral DNA or its integration or both. We investigated the synthesis of unintegrated proviral DNA, using several clones of B-, N-, or NB-tropic Friend murine leukemia virus. Our results indicate that the accumulation of B-tropic proviral DNA in NIH cells may be inhibited at either the level of linear (Form III) or covalently closed circular DNA (Form I), depending upon the degree of restriction of the clone of virus used. We confirmed that there is an effect of the Fv-1 gene on the accumulation of Form I DNA of either B- or N-tropic Friend murine leukemia virus. However, the decrease in infectious centers effected by the Fv-1 gene did not correlate quantitatively with the effect on Form I proviral DNA produced by N-tropic Friend murine leukemia virus in nonpermissive cells. Lastly, we demonstrated in nonpermissively infected NIH cells that a rapidly migrating doublet of viral DNA is formed. (Author abstract) (27 Refs)

24. LYMPHOID CELL TRANSFORMATION BY ABELSON MURINE LEUKEMIA VIRUS (MEETING ABSTRACT).

Baltimore D
Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, 02139
Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

When bone marrow or fetal liver cells are exposed in culture to Abelson murine leukemia virus (A-MulV) the virus induces the formation of colonies of immortalized lymphoid cells. The phenotype of these cells is variable but the established protein markers in the cell indicate that they are related to pre-B-lymphoid cells. The virus is able to transform these cells by virtue of a protein synthesized under the direction of the virus. The viral RNA is evolved partly from a normal cellular gene and the protein formed by the virus is a hybrid of virus-specific and cell-specific polypeptide sequence. This polypeptide has associated with it protein kinase activity that forms a phosphotyrosine bond between the A-MuLV protein itself and the gamma-phosphate derived from ATP. We have analyzed the structure of the normal cell gene corresponding to the A-MuLV cellular insert and know that it has a specific chromosomal location and a structure including a number of exons and introns. A large segment of the normal cell gene has been cloned and is under detailed investigation. Properties of the A-MuLV protein suggest that it is related to pathways used in the cell to respond to hormonal stimulations. (3 Refs)

25. CELLULAR HOMOLOGUE (C-SRC) OF THE TRANSFORMING GENE OF ROUS SARCOMA VIRUS: ISOLATION, MAPPING, AND TRANSCRIPTIONAL ANALYSIS OF C-SRC AND FLANKING REGIONS.

Parker RC, Varmus HE, Bishop JM
Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143
Proc Natl Acad Sci USA; 78(9,part2):5842-5846 1981

The tumorigenic properties of Rous sarcoma virus are attributable to a 60,000-dalton protein, pp60v-src, encoded by a single viral gene, v-src, A homologous gene, c-src, that contains the information for a 60,000-dalton protein, pp60c-src, has been identified in all tested vertebrate cells. By screening a recombinant DNA library representative of the chicken genome, we isolated two overlapping DNA fragments that contain more than 30 kilobases (kb) of DNA spanning the coding sequences for pp60c-src. This 30-kb region is devoid of moderately or highly repeated sequences and shares homology with the entire viral gene and noncoding sequences 5' of v-src. Although v-src has an uninterrupted coding sequence, c-src is interrupted by a minimum of seven intervening sequences. At least two polyadenylylated RNAs seem to be encoded within the DNA we have isolated. The larger RNA, approx 3.9 kb, is the presumptive c-src mRNA; the smaller transcript of about 2 kb hybridizes to DNA sequences several kb from the 3' end of the v-src/c -src homology area. (Author abstract) (28 Refs)

26. AVIAN LYMPHOID LEUKOSIS IS CORRELATED WITH THE APPEARANCE OF DISCRETE NEW RNAS CONTAINING VIRAL AND CELLULAR GENETIC INFORMATION.

Hayward WS, Neel BG, Fang J, Robinson HL, Astrin SM
Rockefeller Univ., 1230 York Ave., New York, NY, 10021
Haematol Blood Transfus; 26:439-444 1981

The virus-related RNA and DNA from 23 avian leukosis virus (ALV)-induced tumors (22 lymphomas, 1 fibrosarcoma) was analyzed. The normal 35S and 21S ALV RNAs were present in many of the lymphomas analyzed. Of the 22 lymphomas, 19 (from 14/17 birds) contained new RNA species not found in uninfected cells or in nontransformed infected cells. In all cases where more than one tumor from the same animal was examined, the same new tumor-specific RNA was found. Class I and II RNAs were not detectable with circular DNA(cDNA)rep, a probe representing the entire viral genome; this indicated that these RNAs contain little, if any, viral information other than the 5' sequences and presumably contain cellular sequences. Class III RNA was detected but the intensity of the bands suggested that only a small portion of the information in these RNAs could be virus-specific. Three of the four tumors analyzed by liquid hybridization contained low or undetectable amounts of 35S and 21S RNAs. In each of these tumors, the levels of RNA detected with cDNAss was at least 20-fold greater than that detected with cDNArep. The ss-containing RNA, presumably representing the new tumor-specific RNAs, was present at levels ranging from 100-1,300 copies per cell. The expression of cellular transformation-specific sequences was studied. New tumor-specific restriction fragments, containing both viral and cellular sequences (JF: junction fragments), were present in lymphoma DNA. All tumors from one bird contained the same tumor-specific JF. New tumor-specific JF were found in all tumors analyzed. Four size classes of JF were found to be common to more than one tumor. The presence of a specific JF correlated with the presence of a new tumor-specific RNA in 8/10 birds in which both RNA and DNA were analyzed. The data suggested that expression of viral genes is not required for maintenance of neoplasia and that lymphoma induction seems to occur only when the provirus integrates at one of a limited number of sites on the host cell chromosome. (25 Refs)

27. THE MOLECULAR BASIS OF AVIAN RETROVIRUS-INDUCED LEUKEMOGENESIS.

Kung HJ, Fung YK, Crittenden LC, Fadly A, Dube SK
No affiliation given
Haematol Blood Transfus; 26:445-451 1981

The fate of proviral DNA in lymphoid leukosis (LL) induced by avian leukosis virus RAV-1 was studied. Sixteen newborn 151-5 x 7-2 chickens were each infected with 10(5) clone-purified viruses. Bursal specimens were obtained at 4 and 8 wk post infection. Leukosis appeared at 16-20 wk. DNA from p releukosis bursae, tumors, and metastases was analyzed using complementary DNA (cDNA) and restriction endonucleases SacI and EcoRI. Three tumor-specific (TS) bands were identified in gel electrophoresis. TS bands were not present in 4-wk bursal samples, although RAV-1 fragments were, indicating that proviral DNA was integrated into host genome at multiple sites. Samples taken at 8 wk showed greater viral infection. Tumor DNA analysis indicated that only a small number of infected cells produced tumor, and that tumor origin was clonal. Variations in TS bands suggested that integration of proviral DNA at a number of sites could produce tumorigenesis. Deletions from viral genome in tumor DNA were apparent. Preferred integration sites for tumorigenesis were indicated. Metastases showed the same clonal origin as primary tumors. Multiple metastases were derived from the same clonal source, suggesting that only one of several original tumor foci contributed to secondary spread. (14 Refs)

28. TRANSFORMING GENES OF RETROVIRUSES: DEFINITION, SPECIFICITY, AND RELATION TO CELLULAR DNA.

Duesberg PH, Bister K
Dept. Molecular Biology, Wendell M. Stanley Hall, Univ. California, Berkeley, CA, 94720
Haematol Blood Transfus; 26:383-396 1981

In this review, onc genes of the rapidly transforming sarcoma and acute leukemia viruses are described and defined. The question is then pursued as to whether the oncogenic specificity of some and lack of specificity of other viruses is due to distinct onc genes or whether one such gene can cause multiple forms of cancer. An additional question addressed is the relationship between viral onc genes and cellular DNA. The final question dealt with is how lymphatic leukemia viruses, which lack known onc genes, may cause cancer. The focus is mainly on avian tumor viruses, because their genetic structures are better defined than those of other viruses. (83 Refs)

29. VIRAL MECHANISMS OF CARCINOGENESIS (MEETING ABSTRACT).

Weiss RA
Chester Beatty Research Institute, Institute of Cancer Research, Fulham Road, London SW3 6JB, England
Symposium on Host Factors in Human Carcinogenesis. June 8-11, 1981, Cape Sunion, Greece International Agency for Research on Cancer; 1981

Tumor virus genomes may be regarded as transposable genetic elements. By integrating into host cell chromosomal DNA they may cause mutations and chromosomal rearrange-

ments that predispose to cancer. However, there is growing evidence that several strains of DNA and RNA tumor viruses carry genes that encode 'transforming' proteins. The transforming proteins of several oncogenic viruses function as protein kinases. Some of the viral 'oncogenes' are similar to cellular genes, and their normal counterparts may play roles in regulated cell differentiation and proliferation. The origin, nature and functio ns of these proteins will be reviewed. (no Refs)

30. GENOMIC INTEGRATION OF BOVINE LEUKEMIA PROVIRUS AND LACK OF VIRAL RNA EXPRESSION IN THE TARGET CELLS OF CATTLE WITH DIFFERENT RESPONSES TO BLV INFECTION.

Kettmann R, Marbaix G, Mammerickx M, Burny A
Faculte des Sciences Agronomiques de l'Etat, Chaire de Zootechnie, Gembloux, Belgium
Haematol Blood Transfus; 26:495-497 1981

Bovine leukemia virus (BLV) integration sites in DNA preparations from target tissues of BLV-infected animals and viral RNA expression in the same cells were examined. DNA was digested by bacterial restriction endonucleases and submitted to electrophoresis in agarose gels. After transfer to nitrocellulose paper the DNA fragments were annealed to a specific BLV (32 P) cyclic DNA probe. Genomic DNA fragments containing viral information appeared after autoradiographic development as individual bands and sometimes smears. Comparison of BLV provirus integration and viral RNA expression in WBC of asymptomatic BLV carriers (AB+), from animals in persistent lymphocytosis (PL+), and from tumor cases (T+) was made. No BLV proviral sequences were detected in three AB+ cases. Circulating ABC of PL animals appear to accommodate BLV at many possible sites; lymphocytes infected by BLV and found in enzootic bovine leukosis tumors from two animals seemed to constitute monoclonal populations of cells carrying one copy of the proviral genome integrated at one genomic site. Liquid hybridization and BLV (3H) cyclic DNA were used to search for viral RNA sequences in total RNA samples from BLV-infected cell. In 7/8 animals tested, transcription of the integrated viral genome from WBC did not occur or occurred at a low level (5.8-8.6% hybridization). Tumor cells gave similar results. (3 Refs)

31. ENDOGENOUS TYPE C RETROVIRAL SEQUENCES OF MICE ARE ORGANIZED IN A SMALL NUMBER OF VIRUS-LIKE CLASSES AND HAVE BEEN ACQUIRED RECENTLY.

Dolberg DS, Bacheler LT, Fan H
Div. Biology and Medicine, Lawrence Berkeley Lab., Univ. California, Berkeley, CA, 94720
J Virol; 40(1):96-106 1981

We studied endogenous Type C virus-related sequences of mice by annealing Moloney murine leukemia virus DNA to agarose gel blot transfers of uninfected mouse cell DNA which had been cleaved with restriction enzymes. We found that many of the endogenous murine leukemia virus-related sequences in mice consist of two organizational classes that are integrated into many different loci. Both of these classes resemble standard murine leukemia virus proviral DNA in both size and sequence organization. All lines of inbred mice examined contained both organizational classes, as did feral isolates of Mus musculus domesticus. However, a related Asian subspecies, Mus musculus molossinus, contained different organizational classes of endogenous murine leukemia virus-related sequences. Among inbred strains and feral isolates of M. musculus domesticus, the murine leukemia virus-related sequences were present at different loci. This suggested that most of these sequences were acquired relatively recently during subspeciation and inbreeding. (Author abstract) (38 Refs)

32. THE ENDOGENOUS PROVIRAL MOUSE MAMMARY TUMOR VIRUS GENES OF THE GR MOUSE ARE NOT IDENTICAL AND ONLY ONE CORRESPONDS TO THE EXOGENOUS VIRUS.

Herrlich P, Hynes NE, Ponta H, Rahmsdorf U, Kennedy N, Groner B
Institut fur Genetik und fur Toxikologie, Kernforschungszentrum Karlsruhe, Postfach 3640, D 7500 Karlsruhe 1, W. Germany
Nucleic Acids Res; 9(19):4981-4995 1981

The endogenous proviral copies of mouse mammary tumor virus (MMTV) were selected from a gene library of GR mouse DNA. We obtained five different lambdaMMTV recombinant clones. Four of them correspond to the 3' Eco RI fragments of the endogenous proviruses and one comprises an intact MMTV provirus with 2 to 3 kb of flanking mouse genomic DNA. Heteroduplex formation followed by S1 digestion under stringent conditions shows that there is nucleotide sequence heterology among the cloned endogenous proviral copies. Only one endogenous proviral copy, associated with the mtv-2 locus, was found to be totally homologous to the exogenous proviral DNA. (Author abstract) (22 Refs)

33. CLONED MOUSE MAMMARY TUMOR VIRUS DNA IS BIOLOGICALLY ACTIVE IN TRANSFECTED MOUSE CELLS AND ITS EXPRESSION IS STIMULATED GLUCOCORTICOID HORMONES (MEETING ABSTRACT).

Buetti E, Diggelmann H
Institut Suisse de Recherches Experimentales sur le Cancer, CH-1066 Epalinges, Switzerland
Experientia; 37(6):640 1981

We have cloned circular unintegrated mouse mammary tumor virus (MMTV) DNA from infected rat hepatoma cells in bacteriophage lambda. Seven independent clones containing MMTV DNA of homogeneous length of 9 kilobase (kb) (5) or 10 kb (2) were identified. The 5 9-kb clones had identical restriction maps consistent with that of 9 kb unintegrated DNA, the other 2 were aberrant. MMTV DNA inserts were purified, ligated and used for cotransfection of Ltk- cells together with a plasmid containing the thymidine kinase gene of herpes simplex virus. All Tk+ cell clones acquired new MMTV sequences and those transfected with the 9 kb MMTV DNA synthesized normal viral RNA and proteins. Viral gene expression was increased by the addition of dexamethasome. (no Refs)

34. DNA SEQUENCE ANALYSIS OF THE LARGE TERMINAL REPEAT OF MOUSE MAMMARY TUMOR VIRUS (MMTV) DNA (MEETING ABSTRACT).

Fasel N, Pearson KW, Buetti E
Institut Suisse de Recherches Experimentales sur le Cancer, CH-1066 Epalinges, Switzerland
Experientia; 37(6):644 1981

Large terminal repeats (LTR) of MMTV DNA might play an important role in the int egration of viral DNA into the host genome. The restriction enzyme fragment containing the LTR might also harbor the sequences necessary for the glucocorticoid sensitivity of viral gene expression. Therefore we decided to sequence this 1.5-kb fragment derived from circular MMTV DNA clones in phage. For this purpose, we subcloned

all of the Pst I fragments in the plasmid pBR 322 prior to sequence analysis. (no Refs)

35. THE LONG TERMINAL REPEAT OF MOLONEY SARCOMA PROVIRUS ENHANCES TRANSFORMATION.

Blair DG, Oskarsson M, McClements WL, Van de Woude GF (c/o Van de Woude), NIH, 9000 Rockville Pike, Bethesda, MD, 20014
Haematol Blood Transfus; 26:460-466 1981

Enhancement by the long terminal repeat (LTR) of Moloney sarcoma virus (MSV) of transformation by an internal MSV fragment containing the MSV-acquired V-mos sequence in mouse cells was reviewed. DNA sequences containing only V-mos transformed NIH 3T3 cells with an activity 10,000-fold lower than that of HT1 MSV DNA. DNA containing only the 5' or 3' LTR of MSV had no transforming activity. When DNA fragments containing LTR were combined with fragments containing V-mos, the full transforming activity of HT1 MSV DNA was obtained. Mutants of lambda-MSV hybrids retained one of two LTR present in MSV, but lost the second LTR and the V-mos sequences. When the retained LTR was cloned as 'pm1sp' or 'pHT1sp' and transfected with DNA containing V-mos, transformation was enhanced in NIH 3T3 cells. Neither pm1sp nor pHT1sp alone produced any transformation. The cellular genome fragment C-mos did not transform cells unless normal Balb/c sequences 5' to C-mos were replaced by MSV sequences, which included LTR and portions of the gag, pol, and env genes. Env apparently was required for expression of C-mos. (18 Refs)

36. FORMATION OF INFECTIOUS PROGENY VIRUS AFTER INSERTION OF HERPES SIMPLEX THYMIDINE KINASE GENE INTO DNA OF AN AVIAN RETROVIRUS.

Shimotohno K, Temin HM
Natl. Inst. Genetics, Misima, 411, Japan
Cell; 26(1):67-77 1981

We have prepared several infectious stocks of an avian retrovirus, spleen necrosis virus, containing the herpes simplex virus type 1 thymidine kinase (tk) gene. The viruses were produced after cotransfection of chicken cells with DNA from recombinants between cloned spleen necrosis virus and tk DNAs and DNA of cloned reticuloendotheliosis virus strain A. Removal of sequences in the tk gene for the end of tk mRNA increased a thousandfold the yield of infectious recombinant virus. Infection of chicken or rat tk- cells with the recombinant virus transformed them to a tk+ phenotype. (Author abstract) (35 Refs)

37. SV40 RECOMBINANT MOLECULES EXPRESS THE GENE ENCODING P21 TRANSFORMING PROTEIN OF HARVEY MURINE SARCOMA VIRUS.

Gruss P, Ellis RW, Shih TY, Konig M, Scolnick EM, Khoury G
Lab. Molecular Virology, NCI, Bethesda, MD, 20205
Nature; 293(5832):486-488 1981

Construction of the simian virus 40 (SV40)-Harvey murine sarcoma virus (Ha-MuSV) recombinants is described. The presence of a Ha-MuSV promoter element preceding the gene encoding p21 was shown. The new promoter element was found to be active not only in cells infected with recombinant but also in cells transformed with Ha-MuSV. (32 Refs)

ANTIVIRALS

38. ANTIRETROVIRAL ACTION OF INTERFERON (MEETING ABSTRACT).

Naso R, Wu Y, Arlinghaus R, Edbauer C
Department of Tumor Virology, University of Texas System Cancer Center, Houston, TX
Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

Mouse fibroblast interferon (IF) inhibited the release of Rauscher leukemia virus from chronically infected NIH Swiss mouse embryo cells by 90-95% at 10 units/ml. The antiretroviral effect was prevented by preincubation of the IF with trypsin or with antiserum of IF or by incubation of the cells with Actinomycin D. IF treatment did not alter the synthesis of any known viral precursor protein; nor did it alter the intracellular cleavage of those precursors into the proteins of mature virions. Furthermore, the viral proteins characteristic of the virus released from IF-treated cells appeared normal with the exception of an extremely small amount of fucosylated glycoprotein precursor, gPr93env, which was incorporated into the envelope of the virus. This precursor-like protein, however, was present in comparable amounts in both control and IF-treated cells. We found, using a radioimmuno-precipitation assay to measure levels of precursor polyproteins in newly infected cells, that virus released by IF-treated cells was nearly as infectious as virus released from normal cells. The above results suggest that IF induces in cells an antiretroviral effect without an apparent alteration in any viral component. It is also suggested that IF induces alterations in cellular structures (eg membranes) so as to alter functions (eg membrane fluidity) which are necessary for the recruitment of viral proteins and/or the clearing of cell proteins at virus assembly sites in the cell membrane. We have not, however, observed any alteration in cell microtubule or microfilament structure (at concentrations of interferon up to 300 units/ml) which would effect the presumed change in membrane fluidity; nor have we observed, at this point in time, any unusual partitioning of viral proteins between cytoplasmic and membrane structures during IF treatment. It remains to be determined whether IF treated of cells induces the synthesis of integral membrane proteins which, when inserted into the cell membranes, alter membrane fluidity and, therefore, virus assembly. (no Refs)

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VIRAL PROTEINS

1. SUGARS ASSOCIATED WITH THE ANTIGENIC REACTIVITY OF THE CARBOHYDRATE MOIETY OF THE GLYCOPROTEIN OF BOVINE LEUKEMIA VIRUS (MEETING ABSTRACT).

Schmerr MF, Miller JM, Van Der Maaten MJ
No affiliation given
Tenth International Symposium for Comparative Research on Leukemia and Related Diseases, August 31-September 4, 1981, Los Angeles, California. 187 pp., 1981.

Unlike other mammalian leukemia viruses, the antibody produced to the major glycoprotein (gp51) of bovine leukemia virus has been found to be directed against the carbohydrate portion of the molecule. Experiments were undertaken to determine which sugars formed the antigenic sites on gp51. The glycoprotein was treated with five different glycosidic enzymes: neuraminidase, beta-galactosidase, alpha-D-mannosidase, beta-N-acetyl glucosaminidase, and alpha-L-fucosidase. Three proteolytic enzymes were also used: proteinase K, Staphylococcus aureus V8 protease, and trypsin. After enzymatic treatment, the glycoprotein was then assayed for its ability to compete with 125Igp51 in a radioimmunoassay. Mannosi-

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dase, fucosidase, and beta-N-acetyl glucosaminidase completely destroyed the antigenic activity of the glycoprotein. Beta-galactosidase reduced the activity of gp51 by approx one-half that of the standard. Neuraminidase slightly enhanced the activity. Addition of either mannosidase, beta-N-acetyl-glucosaminidase or fucosidase to neuraminidase destroyed the antigenic activity. The proteolytic enzymes destroyed antigenic activity as well. Mannose, fucose, and N-acetyl glucosamine appear to be important sugars in determining the antigenic sites of gp51 of bovine leukemia virus. (no Refs)

2. DNA TOPOISOMERASE ACTIVITY ASSOCIATED WITH ROUS SARCOMA VIRUS.

Weis JH, Faras AJ

Dept. Microbiology, Univ. Minnesota Medical Sch., Minneapolis, MN, 55455

Virology; 114(2):563-566 1981

A DNA topoisomerase has been discovered associated with purified virions of Rous sarcoma virus (RSV). The enzyme has been purified from Prague C strain of RSV and compared with the nuclear topoisomerase from chick embryo cells. (Author abstract) (14 Refs)

3. LOCALIZATION OF THE ABELSON MURINE LEUKEMIA VIRUS PROTEIN IN A DETERGENT-INSOLUBLE SUBCELLULAR MATRIX: ARCHITECTURE OF THE PROTEIN.

Boss MA, Dreyfuss G, Baltimore D

Center for Cancer Res., Dept. Biology, Massachusetts Inst. Technology, Cambridge, MA, 02139

J Virol; 40(2):472-481 1981

We examined the interaction of Abelson murine leukemia virus protein P120 with other cellular components after extraction with the nonionic detergent Triton X-100. Most of the Abelson murine leukemia virus P120-associated kinase activity was found in the detergent-insoluble matrix in both lymphoid and fibroblast cell lines. The P120 labeled during a short exposure of cells to [35S]methionine was mainly in the detergent-insoluble matrix (lymphoid cells) or equally distributed in the detergent-insoluble matrix and the soluble fraction (fibroblasts). Steady-state-labeled P120 was distributed equally in the two fractions (lymphoid cells) or mostly in the soluble portion (fibroblasts). Thus, there was an apparent movement of P120 from the detergent-insoluble matrix to the detergent-soluble fraction and a concomitant loss of enzymatic activity. When the detergent-insoluble matrix was incubated with [32P]ATP in situ, phosphorylation of tyrosine residues of P120 was observed. We found an 80,000-mol-wt fragment of P120 (designated F80) after extraction of fibroblast cells with detergent. F80 was not found in extracted lymphoid cells, but mixing labeled lymphoid cells and unlabeled fibroblasts before extraction produced the fragment. F80 contained the gag determinants of P120 but did not react with Abelson-specific serum. These da ta allowed us to assign various features of the protein to regions of the P120 molecule and to localize the Abelson-specific antigenic determinants to the C-terminal region of the molecule. (Author abstract) (29 Refs)

4. CHARACTERISTICS OF AVIAN SARCOMA VIRUS STRAIN PRCIV AND COMPARISON WITH STRAIN PRCII-P.

Breitman ML, Hirano A, Wong T, Vogt PK

(c/o Vogt) Dept. Microbiology, Univ. Southern California, Sch. Medicine, 2011 Zonal Ave., Los Angeles, CA, 90033

Virology; 114(2):451-462 1981

Avian sarcoma virus strain Poultry Research Center IV (PRCIV) is highly oncogenic in chickens and transforms chicken embryo fibroblasts in culture. It is defective in replication, unable to code for complete functional products of the three essential viral genes gag, pol, or env. Nonproducing cells transformed by PRCIV lack the transforming protein of Rous sarcoma virus, pp60src, but synthesize a new transformation-specific protein of 170,000 mol wt. This P170 contains partial gag sequences, probably at its N-terminus and transformation-specific, cell-derived sequences, probably in the C-terminal portion of the molecule. In two-dimensional tryptic peptide maps P170 of PRCIV is indistinguishable from the transformation-specific protein P170 of the previously described PRCII-p. Both P170 proteins also contain all but one tryptic peptide of the transformation-specific gag-linked protein P105 found in cells transformed by avian sarcoma virus PRCII. The P170 proteins of PRCIV and PRCII-p have an associated tyrosine-specific kinase activity similar to P105 of PRCII. All three agents, PRCII, PRCII-p, and PRCIV, belong to Class II of avian sarcoma viruses. In cells transformed by PRCIV or PRCII-p secondary gag-linked transformation-specific proteins of less than 170,000 mol wt are sometimes seen, but limited in vivo and in vitro passage of the viruses shows the P170 to be a stable characteristic of PRCIV and PRCII-p transformation. The genomic RNA of PRCIV and PRCII-p sediments at 30 S corresponding to a size of about 6.1 kilobases. (Author abstract) (35 Refs)

5. AVIAN ACUTE LEUKEMIA VIRUS OK10 HAS AN 8.2-KILOBASE GENOME AND MODIFIED GLYCOPROTEIN GP78.

Pfeifer S, Pettersson RF, Kallio A, Oker-Blom N, Vaheri A

Dept. Virology, Univ. Helsinki, SF-00290 Helsinki 29, Finland

J Virol; 40(2):533-540 1981

We have analyzed the structure of OK10-BM virus, an avian acute leukemia virus produced by a bone marrow-derived cell line of macrophage origin, and compared it with that of OK10 AV, an associated virus originally present in the OK10 virus stock. The RNAs of OK10-BM virus and OK10 AV had the same mobility in agarose gels, corresponding to 8.0 to 8.5 kilobases, a size considerably larger than that of the transforming component (5 to 6 kb) of most other avian acute leukemia viruses. Fingerprint an alysis showed a close relationship between OK10-BM virus and OK10 AV RNAs. The polypeptide compositions of OK10-BM and OK10 AV viruses were similar except for the envelope glycoproteins. In analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the large envelope glycoprotein of OK10-BM virus migrated at Mr = 78,000 (gp78), whereas OK10 AV had the characteristic 85,000-dalton glycoprotein (gp85) of nondefective avian leukemia viruses. gp78 was weakly labeled with methionine, glycine, proline, or mannose, suggesting that purified OK10-BM virus had reduced amounts of the modified envelope glycoprotein. In cell-free rabbit reticulocyte lysates, OK10-BM virion RNA directed the synthesis of a 200,000-dalton polypeptide (p200), a 180,000-dalton polypeptide (pr180), and a 76,000-dalton polypeptide (pr76), whereas OK10 AV RNA gave rise only to pr180 and pr76, suggesting that p200 may represent an OK10-

BM-encoded transforming protein. No biochemical evidence for the presence of an associated helper virus was found in the OK10-BM virus population produced by the macrophage cell line. However, when OK10-BM virus was serially passaged in chicken embryo fibroblasts a virus having structural properties similar to those of OK10 AV (OK10 AV-specific oligonucleotides and gp85) appeared after three passages. Moreover, nonproducer clones of transformed cells could be readily obtained in OK10-BM virus-infected quail cell cultures. It is thus likely that the bone marrow-derived macrophage cell line produces a transforming virus defective in its env gene and low amounts of an associated helper virus, which upon transfer to fibroblasts is preferentially replicated. (Author abstract) (39 Refs)

6. RELATIONSHIP OF THE FELINE ONCORNAVIRUS ASSOCIATED CELL MEMBRANE ANTIGEN TO A FELINE SARCOMA VIRUS ENCODED POLYPROTEIN.

Snyder HW, Dutta-Choudhury M, Hardy Jr WD
No affiliation given
Haematol Blood Transfus; 26:488-491 1981

Using immunoaffinity chromatography on a column of Sepharose containing bound IgG from cat anti-feline oncornavirus-associated cell membrane antigen (FOCMA) serum, a 70,000-dalton protein was isolated from lysates of mink cells transformed by the Gardner-Arnstein strain of feline sarcoma virus (GA-FeSV). This result was unanticipated because previous evidence had suggested that FOCMA was associated with the much larger (95,000 dalton) gag-x fusion protein in these cells. The GA-FeSV gag-x protein was also isolated by virtue of its binding to anti-FeSV antibodies on a different affinity column. Preliminary biochemical characterization of the 70,000-dalton protein from both GA-FeSV-transformed mink fibroblasts and feline lymphosarcoma cells showed no differences between them. The gag-x protein was distinguishable by labelling with 32P-orthophosphate, association with protein kinase activity, and its preci pitation ability with antibodies in a hyperimmune antiserum to FeLV structural proteins. It is hypothesized that FOCMA may actually be a family of antigens, not all of which cross-react with FeSV polyprotein antigens. (21 Refs)

7. TUMOR ASSOCIATED ANTIGENS AND TRANSFORMATION PROTEINS OF FELINE RETROVIRUSES (MEETING ABSTRACT).

Essex M, Chen AP, Mikami T
No affiliation given
Tenth International Symposium for Comparative Research on Leukemia and Related Diseases, August 31-September 4, 1981, Los Angeles, California. 187 pp., 1981.

Cats naturally exposed to feline leukemia virus (FeLV) or feline sarcoma virus (FeSV) mount a humoral antibody response that is directed to tumor associated antigens. Antigens thus far identified as targets for this immunosurveillance-correlated response that are distinct from the virion structural proteins include an antigen of about 70,000 daltons on lymphoid tumor cells (FOCMA-L) and a polyprotein of 84,000 daltons or 110,000 daltons, depending on the strain of virus, on FeSV-transformed fibroblasts. Healthy cats that are exposed to FeLV under natural conditions in leukemia cluster households develop high titers of humoral antibodies to FOCMA. Both adult and young cats that are introduced to such FeLV-exposure environments usually seroconvert to develop FOCMA antibodies within a few mo. When all FeLV-excretor cats are removed from such environments no additional seroconversions occur. However, cats that already have significant humoral antibody titers at the time FeLV-excretor cats are removed maintain humoral antibody titers without re-exposure to FeLV for at least several yr. This suggests that such cats either maintain FeLV in a latent, but not a widely dispersed or excreted form, or that such cats harbor a population of FeLV-altered FOCMA positive cells that are maintained at low levels by an immunosurveillance response. The antigen found in fibroblasts transformed with FeSV is representative of a class of 'gag-x' proteins which are typical of many defective transforming retroviruses in that they contain gag peptides linked to a transformation specific portion designated 'x'. The 'x' portion is encoded by 'one' or 'fes', a member of the set of highly conserved cell genes that become inserted into replication defective retroviruses when they acquire the ability to transform fibroblasts in vitro. Cats inoculated with their own FeSV-transformed cells following in vitro cultivation and transformation develop a humoral antibody response to 'x' of 'gag-x', as do some highly immune cats that have apparently only been exposed to FeLV. When FeSV is used to induce widely different types of tumors, such as melanomas of neuroectodermal origin and fibrosarcomas of mesodermal origin, the same 'gag-x' is expressed concordantly with the malignant phenotype. (3 Refs)

8. PROPERTIES OF A SARCOMA-GROWTH-FACTOR-LIKE PEPTIDE FROM CELLS TRANSFORMED BY A TEMPERATURE-SENSITIVE SARCOMA VIRUS.

De Larco JE, Preston YA, Todaro GJ
Lab. Viral Carcinogenesis, NCI, NIH, Bethesda, MD, 20205
J Cell Physiol; 109(1):143-152 1981

Peptides resembling sarcoma growth factor (SGF) that were produced by virally transformed cells in vitro were characterized. Normal rat kidney (NRK) cells and 3T3 mouse cells were transformed by Moloney sarcoma virus (MSV) or Kirsten sarcoma virus (KiSV). SGF-like factors were obtained from serum-free conditioned media from the cell cultures. Nontransformed cells produced no such factors. The major activity, regardless of cell species or virus type, was produced by a factor with an apparent mol wt of 10,000. Carboxymethyl cellulose chromatography produced similar elution profiles in all cases. Growth factor produced by NRK cells transformed by wild-type KiSV had similar temperature sensitivity to growth factor produced by NRK cells transformed by a temperature sensitive mutant of KiSV. Neither growth factor was temperature-sensitive under the conditions of the assay. THE SGF-like factors produced by transformed cells are apparently produced by cellular genes that are not expressed in the absence of virus. (21 Refs)

VIRAL RNA

9. CHEMICAL METHYLATION OF RNA AND DNA VIRAL GENOMES AS A PROBE OF IN SITU STRUCTURE.

Yamakawa M, Shatkin AJ, Furuichi Y
Roche Inst. Molecular Biology, Nutley, NJ, 07110
J Virol; 40(2):482-490 1981

We used [methyl-3H]dimethyl sulfate to probe the genome structures of several RNA and DNA viruses. We compared sites of modification in nucleic acids that were methylated chemically before and after extraction from purified virions. With both single-stranded and double-stranded substrates alky-

lation occurred mainly at the N7 position of guanine. However, adenine N1 atoms were differentially accessible in single-stranded RNA and DNA. For example, the ratios of 1-methyladenosine to 7-methylguanosine for reovirus mRNA and deproteinized genome RNA were 0.43 and 0.03, respectively. Members of the Reoviridae methylated in situ yielded RNAs with ratios of 0.04 to 0.08, indicating that the intravirion genomes were double stranded. We obtained ratios of 0.26 and 0.35 for the RNAs of dimethyl sulfate-treated brome mosaic and avian sarcoma virions, respectively, which was consistent with partial protection of adenine N1 sites by structural proteins or genome conformation or both. The ratios of 1-methyladenosine to 7-methylguanosine for vaccina virus DNAs methylated in situ (0.10) and after phenol extraction (0.14) were less than the ratios for phiX174 and M13 DNAs (0.39 to 0.64) but considerably greater than the ratio observed with adenovirus DNA (0.002 to 0.02). The presence of a single-stranded region(s) in the vaccinia virus genome was confirmed by S1 nuclease digestion of [methyl-3H] DNA; the released radiolabeled fraction had a ratio of 0.41, compared with 0.025 for the residual duplex DNA. In addition to the structure-dependent accessibility of adenine N1, methylation of adenine N3 was severalfold lower in the intravirion genomes of vaccinia virus, phiX174, and adenovirus than in the corresponding extracted DNAs. Chemical methylation of virions and subviral particles should be useful for in situ analyses of specific regions of RNA and DNA genomes, such as the sites of protein binding during virus maturation. (Author abstract) (30 Refs)

10. LINEAR DENSITY OF NUCLEIC ACID MOLECULES AS A FUNCTION OF DENATURING AND SPREADING CONDITION IN ELECTRON MICROSCOPIC ANALYSIS.

Korb J, Stokrova J, Heine UI
Inst. Molecular Genetics, Czechoslovak Acad. Sciences, Flemingovo n. 2, CS-166 10 Prague 6, Czechoslovakia
Stud Biophys; 84(3):195-206 1981

The behavior of single stranded PhiX 174 DNA, 18S and 28S ribosomal RNA, and avian myeloblastosis (AMV) and vesicular stomatitis (VSV) viral RNA was analyzed under different denaturing and spreading conditions during electron microscopic visualization. Contour lengths and apparent linear density of the DNA and RNAs depended on both denaturation and spreading conditions. (28 Refs)

11. GENOMIC CHARACTERIZATION OF FBJ, FBR, AND RFB MURINE BONE TUMOR VIRUSES.

Rupprecht KR
University of Notre Dame, Notre Dame, IN
Diss Abstr Int [B]; 42(3):920-B 1981

The RNA extracted from virus particles banding at 1.16 g/cc in sucrose was analyzed for intact and subunit genome size by rate zonal centrifugation. The intact genome was found to sediment with a rate constant of 70S for all three viruses. The 70S genome could be dissociated to produce 28S and 34S subunits for FBJ MuSV and FBJ MuLV respectively, 31S and 35S for FRB MuSV and FBR MuLV respectively, and 34S for RFB. Comparative and competitive hybridization demonstrate that FBJ, FBR, and RFB are all closely related to each other. These three viruses were also found to contain sequence homologies with other murine type-C viruses, while no homology was found between the bone tumor viruses and the non-murine or type-B and D viruses examined. The presence of proviral sequences in host strain whole mouse embryo DNA was detected and quantified using DNA-DNA association kinetics. The complete viral genomes of all three viruses were found to be present in the embryonic genomes of their respective host strains, indicating that these viruses are endogenous to these mouse strains. The FBJ and FBR viral genomes were found to be present in the DNA of the CF1 and X/Gf strains respectively at copy numbers of 2-3 copies per haploid mouse genome. The RFB viral genome was found to be present in CF1 DNA at 3-4 copies per haploid mouse genome. While a sarcoma speci fic probe for FBJ MuSV was not produced, a probe was prepared which could differentiate between RNA from the FBJ MuSV complex and RNA from isolated FBJ MuLV. This probe also hybridized extensively to FBR MuSV and RFB RNA's. (no Refs)

12. THE EXPRESSION OF VIRUS-SPECIFIC NUCLEOTIDE SEQUENCES IN ROUS SARCOMA CELLS.

Ratovitaki EA, Shaposhnikov JD, Bobrov YF, Kuznetsov OK
Petrov Res. Inst. Oncology, USSR Public Health Ministry, Leningrad, 188 646, USSR
Neoplasma; 28(4):413-422 1981

Poly(A)-enriched virus-specific mRNAs (v-mRNA) are revealed in the subcellular fractions (nuclei, mitochondria, polyribosomes and cytosol) from chicken and hamster Rous sarcoma cells. The qualitative and quantitative differences in v-mRNA populations from permissive and non-permissive tumors are shown. The sedimentation analysis of v-mRNAs allowed to ascertain the presence of three molecular classes of v-mRNA in nuclei and cytoplasm of chicken Rous sarcoma cells (35S, 28S and 22S, and 35S, 26-28S and 20S, respectively). While two size classes (33S and 20S) are shown in hamster Rous sarcoma nuclei, only 24S v-mRNa is revealed in the cytoplasm of its tumor tissue. Thus, the oncovirus gene expression is restricted in the non-permissive tumor cells, unlike that of the permissive chicken Rous sarcoma cells, both in the level of transcription, and in the processing and transfer v-mRNAs from nucleus to cytoplasm. The virus gene function peculiarities in the tumors of birds and mammals are discussed. (Author abstract) (32 Refs)

13. ANALYSIS OF VIRAL RNA AND PROTEINS EXPRESSED BY A NON PRODUCER FRIEND ERYTHROLEUKEMIA CELL LINE (HFL/B CELL LINE).

Mathieu-Mahul D, Robert J, Gisselbrecht S, Barque JP, Larsen CJ
Laboratoire de Pharmacologie Experimentale, Centre Hayem, Hopital Saint-Louis, 2 place du Docteur Alfred-Fournier, 75475 Paris Cedex 10, France
Leuk Res; 5(4/5):399-411 1981

To study simultaneously viral RNA and protein expression in Friend cells, an erythroblastic cell line (HFL/b) established from a tumor derived from a BALB.B H-2b mouse infected by Polycythemia Friend virus was examined. In contrast with all the previously described HFL/b cell line data, these HFL/b non-producer cells were actually glycoprotein (gp)70-but still conserved an intact spleen focus-forming virus (SFFV) genome. HFL/b cells did not express a 38S RNA with the size of the helper virus genomes but still synthesized 32S SFFV RNA. HFL/b cells did not contain detectable levels of gp70 or its envelope gpr85 precursor but expressed only two 50,000 and 52,000 dalton gp70-related molecules, which are likely to represent the gp50-55 encoded by SFFV. These molecules are glycosylated and are referred to as SFFV gp50 and gp 52. When SFFV gp50 and gp52 were labeled with different sugars, they both incorporated mannose, while galactose w as largely present in a diffuse band moving at the same position as that of the 60-65 K material labeled with 3H-glucosamine. Gag-related polypeptides in HFL/b cell extracts were characterized. The

glycosylation of gag p48 and p50 was studied. Mannose was not incorporated in any other material immunoprecipitated by anti-Rauscher p15 and p12 goat sera, in contrast to glucosamine and galactose, which were present in heterogeneous products migrating in the region of 60,000-65,000 dalton molecules. Neither gag p37 + gag p39 nor gag p58 were recovered from the sugar-labeled HFL/b cell extracts showing that these molecules were not glycosylated at all. (43 Refs)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

14. RESTRICTION OF REPLICATION OF ROUS SARCOMA VIRUS MEDIATED BY THE SRC REGION OF THE GENOME: ANALYSIS OF THE FORMATION AND INTEGRATION OF VIRAL DNA AND THE EFFECTS OF CONDITIONAL AND NONCONDITIONAL MUTATIONS IN SRC.

Neiman PE, Stone J, Gilbert L, Fincham VJ, McMillin-Helsel C, Wyke JA

Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104

Virology; 114(2):516-525 1981

The growth of most Rous sarcoma viruses (RSV) is severely restricted on MSB-1 cells (a line of chicken T lymphoblasts) in comparison to growth on chicken embryo fibroblasts (CEF). Nonconditional transformation defective mutants of RSV from which the complete src region has been deleted (td RSV) are not subject to growth restriction. We examined the formation and integration of RSV and td RSV in MSB-1 cells following high multiplicity infection. Nearly equivalent quantities of the linear form of unintegrated RSV and td RSV DNA were formed in these cells during the first 10 hr after infection. Linear RSV DNA from MSB-1 cells could not be distinguished from linear RSV from CEF by restriction endonuclease analysis and by previously described transfection assays. Beyond 10 hr after infection, and with progressive cell growth in the MSB-1 cultures, the level of RSV linear DNA rapidly decreased. Presumptive circular RSV DNA was detected only transiently, and at very low levels, about 15 hr after infection. Association of RSV DNA with high-mol wt chromosomal DNA, ie, integration, was not detected in this study. In contrast, nearly constant levels of td RSV unintegrated linear DNA and, after 20 hr, circular DNA persisted in MSB-1 cells for at least 7 days after infection. Integration of td RSV proviral DNA was inefficient, occurring in only about 5% of MSB-1 cells (even at very high multiplicities of infection) in the first round of infection, and in 25-40% of cells by 3 days after infection. Almost all MSB-1 cells contained td RSV DNA produced virus. Analysis of eight nonconditional transformation defective mutants of RSV which retain the src region to different extents showed that all of these mutants replicated to the same normal titer on MSB-1 cells as on CEF witho ut further deletion of the src region. Two temperature sensitive src mutants of RSV showed similar growth restriction on MSB-1 cells at both 35 C and 41 C, indicating that thermal inactivation of the src gene product could not abrogate the replication block. These studies clearly demonstrate that the presence of the src region in RSV impedes the formation and/or integration of provirus in some types of host cells. (Author abstract) (24 Refs)

15. EFFECTS OF APHIDICOLIN ON RETROVIRUS DNA SYNTHESIS IN VIVO.

Hagino-Yamagishi K, Kano K, Mano Y

Dept. Physiological Chemistry and Nutrition, Univ. Tokyo, 7-3-1-Hongo, Bunkyo-ku, Tokyo, Japan

Biochem Biophys Res Commun; 102(4):1372-1378 1981

Aphidicolin (AC) markedly reduced progeny virus production in SC-1 cells newly infected with AKR-, Moloney-, and Friend-murine leukemia virus. The results of hybridization analysis of virus-specific RNA indicated that once viral DNA was integrated into the host cell genome, the transcription of viral DNA and progeny virus production were not inhibited by the drug, suggesting that the site of inhibition of AC exists in the process prior to the integration of viral DNA. The results of further experiments in which unintegrated viral DNA from AKR-virus-infected and uninfected SC-1 cells were analyzed by 1% agarose gel electrophoresis and by the Southern blotting technique indicated that DNA from infected cells with AC treatment lacked the large closed circular DNA, while the linear and the small closed circular DNA were still present; these experiments indicated that AC inhibition takes place during the formation of large closed circular DNA. The hybridization patterns of linear DNA fragments obtained by restriction endonuclease digestion were not altered by AC treatment of the cells. The same results were obtained by other restriction endonuclease digestions. Thus, AC apparently inhibits the conversion from linear DNA to large closed circular DNA. (14 Refs)

16. INTEGRATION OF ROUS SARCOMA VIRUS DNA INTO CHICKEN EMBRYO FIBROBLASTS: NO PREFERRED PROVIRAL ACCEPTOR SITE IN THE DNA OF CLONES OF SINGLY INFECTED TRANSFORMED CHICKEN CELLS.

Lerner TL, Skalka AM, Hanafusa H

Rockefeller Univ., New York, NY, 10021

J Virol; 40(2):421-430 1981

We analyzed retroviral integration into a host genome by using avian sarcoma virus infection of natural target cells under conditions where secondary integration via virus spread was inhibited. This was accomplished by using the noninfectious pol-env-alpha variant of the Bryan high-titer strain of Rous sarcoma virus. A total of 12 independent Bryan high-titer Rous sarcoma virus-transformed chicken embryo fibroblast clones were obtained and mapped by using restriction endonucleases. Provirus-cell junction fragments were identified with appropriate hybridization probes. We found that expression of th e viral genes could occur after proviral integration at many sites on the chicken genome and that there was no apparent preference for specific integration sites. (Author abstract) (48 Refs)

17. PURIFIED GLUCOCORTICOID RECEPTORS BIND SELECTIVELY IN VITRO TO A CLONES DNA FRAGMENT WHOSE TRANSCRIPTION IS REGULATED BY GLUCOCORTICOIDS IN VIVO.

Payvar F, Wrange O, Carlstedt-Duke J, Okret S, Gustafsson JA, Yamamoto KR

Dept. Biochemistry and Biophysics, Univ. California, San Francisco, CA, 94143

Proc Natl Acad Sci USA; 78(11,part2):6628-6632 1981

Activated glucocorticoid receptor protein, purified to 40-60% homogeneity from rat liver extracts, binds selectively in

vitro to a cloned fragment of murine mammary tumor virus (MTV) DNA. The DNA fragment tested contains about half of the sequences present in intact MTV DNA, and its rate of transcription, like that of the intact viral element, is strongly stimulated by glucocorticoids when it is introduced into the genome of a receptor-containing cell. In contrast, the receptor fails to bind selectively to DNA restriction fragments from E coli plasmids pBR322 and RSF2124 or from bacteriophages lambda and T4. Preliminary experiments to localize regions within MTV DNA responsible for selective binding have revealed thus far one subfragment that fails to bind the receptor and one selectively bound subfragment that maps far downstream from the 5' terminus of the normal RNA transcript. These studies are consistent with the notion that steroid receptors may modulate rates of transcription by recognizing specific DNA sequences within or near the regulated genes. (Author abstract) (35 Refs)

18. GLUCOCORTICOIDS REGULATE EXPRESSION OF DIHYDROFOLATE REDUCTASE CDNA IN MOUSE MAMMARY TUMOUR VIRUS CHIMAERIC PLASMIDS.

Lee F, Mulligan R, Berg P, Ringold G
Dept. Pharmacology, Stanford Univ. Sch. Medicine, Stanford, CA, 94305
Nature; 294(5838):228-232 1981

Fusions between the mouse mammary tumor virus long terminal repeat and a mouse dihydrofolate reductase cDNA have been constructed in a SV40 vector. When these plasmids are transferred into recipient cells, the production of dihydrofolate reductase is regulated by glucocorticoid hormones. These results define a hormonally responsive region of the viral genome. (Author abstract) (26 Refs)

19. INFECTIVITY AND METHYLATION OF RETROVIRAL GENOMES IS CORRELATED WITH EXPRESSION IN THE ANIMAL.

Stuhlmann H, Jahner D, Jaenisch R
Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie, Universitat Hamburg, Martinistrasse 52, 2000 Hamburg 20, W. Germany
Cell; 26(2,part2):221-232 1981

We studied mechanisms controlling gene expression during animal development using retroviruses as model genes. For this, substrains of mice have been previously derived carrying the Mo loney leukemia virus (M-MuLV) in their germ line. Virus activation occurs in some of these substrains at different stages of development, resulting in two classes of viral genomes. The genetically transmitted (endogenous) copy is present in every cell, whereas somatically acquired ('exogenous') copies are carried only in cells that were superinfected. We compared these two classes of M-MuLV genomes using two parameters. DNA sequences of the endogenous M-MuLV genome in all mouse substrains were highly methylated in GCGC, the recognition sequence of the restriction enzyme Hha I, and were not infectious (specific infectivity less than 10(-7) pfu per proviral genome) in a DNA transfection assay. In contrast, the 'exogenous' copies were hypomethylated and infectious. These parameters are strongly correlated to genome activity in the animal: only tissues carrying exogenous copies express virus-specific RNA. With the assumption that gene expression of transfected DNA is controlled by mechanisms that are relevant for gene expression in the animal, our results suggest that DNA methylation plays a causative role in gene regulation during development and differentiation. (Author abstract) (43 Refs)

20. DNA SEQUENCES HOMOLOGOUS TO VERTEBRATE ONCOGENES ARE CONSERVED IN DROSOPHILA MELANOGASTER.

Shilo BZ, Weinberg RA
Dept. Virology, Weizmann Inst., Rehovot, Israel
Proc Natl Acad Sci USA; 78(11,part2):6789-6792 1981

Sequences homologous to the oncogene sequences of acute RNA tumor viruses have been shown to be highly conserved within vertebrates. In the present work, eight different oncogene DNA sequences have been used as probes to search for homologous sequences in the DNA of organisms of other phyla. Five of these probes hybridized to the DNA of Drosophila melanogaster. Abelson leukemia virus probe detected a single homologous DNA fragment in Drosophila DNA. In contrast, probes prepared from the genomes of Harvey, avian, and feline sarcoma viruses and avian myelocytomatosis virus hybridized with multiple homologous sequences in Drosophila DNA. The identification of sequences homologous to vertebrate oncogenes in invertebrates demonstrates both a high degree of conservation of these genes and a wide distribution among divergent species. It seems likely that sequences homologous to vertebrate oncogenes play a crucial role in metazoan metabolism. (Author abstract) (31 Refs)

21. STRUCTURAL ORGANIZATION AND BIOLOGICAL ACTIVITY OF MOLECULAR CLONES OF THE INTEGRATED GENOME OF A BALB/C MOUSE SARCOMA VIRUS.

Andersen PR, Tronick SR, Aaronson SA
Lab. Cellular and Molecular Biology, NCI, Bethesda, MD, 20205
J Virol; 40(2):431-439 1981

BALB/c mouse sarcoma virus (BALB-MSV) is a spontaneously occurring transforming retrovirus of mouse origin. The integrated form of the viral genome was cloned from the DNA of a BALB-MSV-tr ansformed nonproducer NRK cell line in the Charon 9 strain of bacteriophage lambda. In transfection assays, the 19-kilobase-pair (kbp) recombinant DNA clone transformed NIH/3T3 mouse cells with an efficiency of 3 x 10(4) focus-forming units per pmol. Such transformants possessed typical BALB-MSV morphology and released BALB-MSV after helper virus superinfection. A 6.8-kbp DNA segment within the 19-kbp DNA possessed restriction enzyme sites identical to those of the linear BALB-MSV genome. Long terminal repeats of approx 0.6 kbp were localized at either end of the viral genome by the presence of a repeated constellation of restriction sites and by hybridization of segments containing these sites with nick-translated Moloney murine leukemia virus long terminal repeat DNA. A continuous segment of at least 0.6 and no more than 0.9 kbp of helper virus-unrelated sequences was localized toward the 3' end of the viral genome in relation to viral RNA. A probe composed of these sequences detected six EcoRI-generated DNA bands in normal mouse cell DNA as well as a smaller number of bands in rat and human DNAs. These studies demonstrate that BALB-MSV, like previously characterized avian and mammalian transforming retroviruses, arose by recombination of a Type C helper virus with a well-conserved cellular gene. (Author abstract) (44 Refs)

22. VERTEBRATE DNAS CONTAIN NUCLEOTIDE SEQUENCES RELATED TO THE TRANSFORMING GENE OF AVIAN MYELOBLASTOSIS VIRUS.

Bergmann DG, Souza LM, Baluda MA
Univ. California Los Angeles Sch. Medicine, Los Angeles, CA, 90024
J Virol; 40(2):450-455 1981

Avian myeloblastosis virus contains a continuous sequence of approx 1,000 nucleotides which may represent a gene (amv) responsible for acute myeloblastic leukemia in chickens. This sequence appears to have been acquired from chicken DNA and to be substituted for the envelope gene in the viral genome. We used hybridization probes enriched for the amv sequences and conditions that facilitate annealing of partially homologous nucleotide sequences to show that cellular sequences related to amv are present in the genomes of all vertebrates ranging from amphibians to humans but were not detected in fish, sea urchins, or Escherichia coli. In contrast to the preceding findings, nontransforming endogenous proviral nucleotide sequences closely related to the remainder of the avian myeloblastosis virus genome and to the entire myeloblastosis-associated helper virus are present only in chicken DNA. The amv-related cellular sequences appear to be highly conserved during evolution and to be contained at only one or a few locations in the genome of vertebrates. Within closely related species, they appear to share common evolutionary genetic loci. These findings and similar ones obtained with other highly oncogenic retroviruses containing a tran sforming gene suggest a general mechanism for acquisition of viral oncogenic sequences and an essential role for these sequences in the normal cellular state. (Author abstract) (29 Refs)

23. EXPERIMENTS WITH CLONED COMPLETE TUMOR-DERIVED BOVINE LEUKEMIA VIRUS INFORMATION PROVE THAT THE VIRUS IS TOTALLY EXOGENOUS TO ITS TARGET ANIMAL SPECIES.

Deschamps J, Kettmann R, Burny A
Dept. Molecular Biology, Univ. Brussels, B-1640 Rhode St-Genese, Belgium
J Virol; 40(2):605-609 1981

Taking advantage of the existence of a unique SacI restriction site in the long terminal repeats of the integrated bovine leukemia virus proviral DNA isolated from a bovine tumor, the total viral information (about 9.2 kilobases) was cloned in the lambdoid vector lambdaWES.lambdaB. Use of this cloned bovine leukemia virus DNA allowed us, for the first time, to definitely rule out the existence of any endogenous bovine leukemia virus sequence in the bovine, ovine, caprine, murine, feline, chicken, or human genomes. These data prove the absence of acquired cellular information in the provirus that has given rise to a tumor. (Author abstract) (20 Refs)

24. COMPARISON BETWEEN THE VIRAL TRANSFORMING GENE (SRC) OF RECOVERED AVIAN SARCOMA VIRUS AND ITS CELLULAR HOMOLOG.

Takeya T, Hanafusa H, Junghans RP, Ju G, Skalka AM
Rockefeller Univ., New York, NY, 10021
Mol Cell Biol; 1(11):1024-1037 1981

To gain information on the relationship between the src region of Rous sarcoma virus (RSV) (v-RSV) and cellular src (c-src), and clues to the origins of recovered avian sarcoma viruses (rASV), molecular clones were isolated containing src related sequences from the parental RSV, from rASV, and from c-src, the presumed progenitor of rASV. Molecular clones of viral DNA from rASV and its transformation-competent progenitor, the Schmidt-Ruppin A strain of RSV were constructed and analyzed. A 2.0-megadalton EcoRI fragment, containing the entire src gene from each of the clones was subcloned and characterized. The fragments were also used as probes to isolate recombinant phage clones containing the cellular counterpart of the viral src gene (c-src), from a lambda library of chicken DNA. Restriction endonuclease mapping of c-src revealed extensive similarity between the src region of RSV and rAVS, but striking differences between viral src's and c-src. Electron microscopic analysis of heteroduplexes between recovered virus src and c-src revealed a 1.8-kilobase homologous region. In the cellular gene, the homologous region was interrupted by seven nonhomologous regions which are interpreted to be intervening sequences. Minimum length of c-src is estimated at 7.2 kilobases. The findings have implications concerning the mechanism of formation of recovered virus src and possibly other cell-derived retrovirus transforming genes. (58 Refs)

25. THE V-SIS TRANSFORMING GENE OF SIMIAN SARCOMA VIRUS IS A NEW ONC GENE OF PRIMATE ORIGIN.

Wong-Staal F, Dalla Favera R, Gelmann EP, Manzari V, Szala S, Josephs SF, Gallo RC
Lab. Tumor Biology, NCI, Bethesda, MD, 20205
Nature; 294(5838):273-275 1981

Studies on the cloned simian sarcoma virus (SSV) genome are presented which show that a 1.2-kilobase transformation-specific viral onc gene, v-sis, is distinct from other genes and is derived from a woolly monkey which had previously co-habited with a gibbon before it developed sarcoma. A PstI-PstI fragment of the SSV genome that contains approx 250 base pairs (bp) of 5' v-sis sequences and 900 bp of nondefective helper virus (SSAV)-derived sequences were subcloned into the bacteriophage M13. The single-stranded phage DNA was then partially digested with DNase and terminally labeled with 32P. Labeled DNA was hybridized to cellular DNA from tissues of different animals and resultant hybrids were assayed by their resistance to S1 nuclease. Primate DNA hybridized significantly better than DNA from non-primates. Among primates, highest homology was obtained with woolly monkey DNA. The SSAV genome had no detectable homology to primate DNA and its distant homology to rodent DNA could only be monitored by non-stringent hybridization conditions. It is concluded that v-sis originated from woolly monkey DNA. The close relatedness of SSAV to different gibbon ape leukemia virus isolates and the revelation that the woolly monkey that yielded SSV/SSAV co-habited with a gibbon before it developed sarcoma suggests that SSAV was transmitted from gibbon to woolly monkey during that period, and that in a single infection event, SSAV had recovered an onc gene from woolly monkey to generate SSV. To date this is the only onc gene of primate origin. (24 Refs)

ANTIVIRALS

26. 2-L-RHAMNOPYRANOSYL[1,2,4]TRIAZOLO[1,5-A]PYRIDINE. 4' AND 3' OXIDATION PRODUCTS. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS.

Allard P, Dinh TH, Gouyette C, Igolen J, Chermann JC, Barre-Sinoussi F
Unite de Chimie Organique, Institut Pasteur, Equipe de Recherche Associee au CNRS (ERA 927), 75724 Paris, Cedex 15, France
J Med Chem; 24(11):1291-1297 1981

A series of 2-alpha-L-rhamnopyranosylnitro[1,2,4]triazolo[1,5 alpha]pyridine C-nucleosides was synthesized from the condensation of a thioiminoether with nitro-2-pyridylhydrazines. Catalytic reduction afforded the corresponding amino derivative. A 1',2' unsaturated C-nucleoside was also obtained by two different routes. Selective oxidation gave the 3'- and 4'-ketonucleosides. The cytotoxic properties of the nucleosides, as well as their effect on viral transformation and replication, were described. The nitro derivatives inhibit viral replication, but at toxic doses; the introduction of a keto function leads to a product which inhibits the replication of murine leukemia virus (MuLV) at noncytotoxic concentrations. The amino derivatives have no significant antiviral effect. (Author abstract) (26 Refs)

27. SODIUM SELENITE INHIBITION OF THE REPRODUCTION OF SOME ONCOGENIC RNA-VIRUSES.

Balansky RM, Argirova RM
Inst. Oncology, Medical Acad., Sofia-1156, Bulgaria
Experientia; 37(11):1194-1195 1981

In mice infected by Rauscher leukemia virus, the addition of sodium selenite to the drinking water had an inhibitory effect on spleen growth. Sodium selenite, when added to bovine leukemia virus in vitro, markedly depressed reverse transcriptase activity. The in vitro results may provide a key to explain the in vivo observations. (Author abstract) (12 Refs)

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- **VIRAL RNA** 7-8
- **REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA** 9-15

VIRAL PROTEINS

1. BIOCHEMICAL CHARACTERIZATION OF MURINE BONE MARROW GP71.

Rubio N
'Centro de Inmunologia Experimental y Aplicada' INMAT, Bolivia, 25, Vigo, Spain
FEBS Lett; 135(1):43-48 1981

AKR-like glycoprotein 71 (gp71) purified from mouse bone marrow was studied and compared structurally with some viral proteins. The purification of bone AKR-murine leukemia virus (AKR-MuLV) gp71 is described. Immunoprecipitation was performed on both viral and bone marrow gp71. Tryptic peptide maps of bone marrow, AKR-MuLV and Balb (X) gp71 are presented. A distinct pattern of spot distribution was observed in each case. The comparison of the bone marrow antigens with both viral products showed that the tissue gp71 resembles the xenotropic map more than the AKR map; the NIH Swiss mouse bone marrow protein also resembled the xenotropic map. Thus the antigenic relation was not accompanied by clear structural similarity. Bone marrow antigen and viral gp71 showed similar elution behavior in Sepharose columns and migrated to the same position in sodium dodecyl sulfate-polyacry-

HE 20.3173/2:

lamide gels suggesting that the bone marrow antigen is also a glycoprotein of 70,000-71,000 mol wt. The AKR gp-71-like antigen accounted for 180 and 140 nanog/mg total bone marrow protein in Balb/c and NIH Swiss mice, respectively. (14 Refs)

2. ANALYSIS OF TRANSFORMING GENE PRODUCTS FROM MOLONEY MURINE SARCOMA VIRUS.

Papkoff J, Lai MH, Hunter T, Verma IM
Dept. Biology, Univ. California, San Diego, La Jolla, CA, 92093
Cell; 27(1,part2):109-119 1981

We previously showed that in vitro translation of M-MuSV virion RNA yielded a 62 kd gag gene product and an overlapping set of four proteins with approx mol wt of 37,000, 33,000, 24,000 and 18,000. In this paper we show, by use of hybrid arrest translation with cloned recombinant DNAs containing M-MuSV v-mosMo sequences, that the 37, 33, 34 and 18 kd proteins are synthesized in their entirety from the v-mosMo gene. Analysis of the primary sequence of these proteins shows that each one is initiated independently from AUG codons within the v-mosMo gene and utilizes the long open reading frame predicted from the v-mosMo DNA sequence. Antisera against synthetic peptides corresponding to the C terminus of the predicted v-mosMo gene product precipitate all four in vitro v-mosMo proteins. (Author abstract) (39 Refs)

3. ROUS SARCOMA VIRUS TRANSFORMING PROTEIN, P60SRC, EXPRESSED IN E. COLI, FUNCTIONS AS A PROTEIN KINASE.

Gilmer TM, Erikson RL
Dept. Pathology, Univ. Colorado Health Sciences Center, Denver, CO, 80262
Nature; 294(5843):771-773 1981

Rous sarcoma virus (RSV) p60src produced by Escherichia coli which carry plasmids expressing the src gene was selected by immunoaffinity chromatography and tested for expression of protein kinase (PK) activity. The p60src virus phosphorylated tyrosine residues in tubulin and casein substrates; phosphorylation was inhibited by anti-p60src IgG in a reaction that resulted in phosphorylation of the immune IgG. No PK activity was detectable in bacterial preparations lacking the src gene. Expression of p60src-specific PK activity in E coli suggests that the enzyme activity observed in eukaryotic cells is encoded in the src gene. (25 Refs)

4. IDENTIFICATION OF A 20,000-DALTON PROTEIN IN SSV-TRANSFORMED NONPRODUCER CELLS.

Thiel HJ, Matthews TJ, Weinhold KJ, Broughton EM
Bundesforschungsanstalt fur Viruskrankheiten der Tiere, P.O. Box 1149 Paul Ehrilch-Strasse 28, Tubingen, W. Germany
Virology; 115(2):401-405 1981

Simian sarcoma virus-transformed nonproducer cells (SSV-NP cells) express a 20,000-dalton protein (p20), which is precipitated by an autologous antiserum against SSV-NP cells. The molecule is shed or secreted from the cells because it was found in cell extracts as well as tissue culture supern atants. P20 was detected in SSV-transformed cells from three different species, but not in helper virus (SSAV)-infected nontransformed cells. The data suggest that the expression of p20 is specific for transformation by SSV. (Author abstract) (14 Refs)

5. RECENT DISCOVERIES ON CELLULAR PROTEINS IN RELATION TO VIRALLY AND CHEMICALLY INDUCED CARCINOGENESIS.

Jornvall H
Kemiska Institutionen I, Karolinska Institutet, S-104 01 Stockholm, Sweden
Lakartidningen; 78(41):3585-3586 1981

Recent studies on the molecular mechanisms of carcinogenesis suggest the existence of a cellular protein ('53K') common to virally and chemically induced cell transformations. This type of protein binds some T-antigens from oncogenic DNA viruses, including large T-antigen from SV40 and Epstein-Barr virus-determined nuclear antigen. Transformation-related proteins from oncogenic RNA viruses are similar to normal cellular proteins. Several of these proteins have phosphorylating activity, and some of them have been found to be protein kinases. Associations of such proteins with the plasma membrane have also been shown. These different connections between various tumor-related proteins and normal cellular proteins suggest the importance of gene determination in transformations. (11 Refs) (In SWE)

6. ROLE OF ONCOGENES IN THE DEVELOPMENT OF TUMORS.

Klein G
Institutionen for tumorbiologi, Karolinska institutet, Stockholm, Sweden
Lakartidningen; 78(41):3575-3577 1981

Recent studies in viral oncogenes are reviewed. The coupling of some normal genes is a common feature of all tumor transformation processes caused by not directly-transforming RNA tumor viruses. Unlike the RNA viruses, the DNA viruses belong to several greatly different groups. Production of the so-called T antigen is a common feature of all DNA tumor viruses. This antigen occurs in all transformed cells. There are at least three virus types (papilloma virus, Epstein-Barr virus (EBV), and hepatitis B virus) contribute to the development of certain human tumors, but their contributions are less direct than in the case of infectious diseases. Only papilloma virus induces tumors directly, and most of them are benign, but papilloma virus may play a certain role in the genesis of some malignant epithelial tumors as well. Hepatitis B virus first causes liver cirrhosis, a precancerous state, the development of the tumor clone being the result of abnormal regeneration. (7 Refs) (In SWE)

VIRAL RNA

7. METABOLISM OF VIRAL RNA IN MURINE LEUKEMIA VIRUS-INFECTED CELLS: EVIDENCE FOR DIFFERENTIAL STABILITY OF VIRAL MESSAGE AND VIRION PRECURSOR RNA.

Messer LI, Levin JG, Chattopadhyay SK
Lab. Molecular Genetics, Natl. Inst. Child Health and Human Development, Bethesda, MD, 20205
J Virol; 40(3):683-690 1981

Molecular hybridization techniques were used to examine the stability of viral message and virion precursor RNA in murine leukemia virus-infected cells treated with actinomycin D. Under the conditions used, viral RNA synthesis was inhibited, but viral protein synthesis continued, and the cells pro-

duced noninfectious particles (actinomycin D virions) lacking genomic RNA. Analysis of total RNA in virions revealed that the amount of hybridizable viral RNA decreased steadily after the addition of actinomycin D and by 8 hr was 10% of the control value. Studies on fractionated viral RNA showed that this low level of hybridization is due to residual 70S RNA in the virion population. The results indicated that viral RNA which is destined to be encapsidated into virions has a half-life of approx 3 to 4 hr. In contrast, other intracellular virus-specific RNA molecules appeared to be quite stable and persisted for a long period of time, with a half-life of at least 12 hr. These observations support the idea that two independent functional pools of 35S viral RNA exist within the infected cell: one serving as message and the other as precursor to virion RNA. The existence of two viral RNA pools was further documented by the finding that 12 hr after the addition of actinomycin D, when virion precursor RNA was depleted, 35S and 21S viral mRNA species could be identified in polyribosomal RNA as well as in total polyadenylated cell RNA. Surprisingly, 35S mRNA declined more rapidly than did 21S mRNA, which appeared to be increased in amount. (Author abstract) (48 Refs)

8. DEXAMETHASONE INDUCTION OF THE INTRACELLULAR RNAS OF MOUSE MAMMARY TUMOR VIRUS.

Robertson DL, Varmus HE
Graduate Section Biochemistry, Dept. Chemistry, Brigham Young Univ., Provo, UT, 84602
J Virol; 40(3):673-682 1981

We have studied the kinetics of dexamethasone induction of mouse mammary tumor virus (MMTV) RNAs and proteins in virus-infected rat XC cells and GR mouse mammary tumor cells. A detectable increase in viral RNA in infected XC cells was present within 10 min after hormone addition, and half-maximal induction was achieved in less than 2 hr. The increase in viral RNA concentration was apparent first in nuclear RNA and later in the cytoplasm. Within the first 15 min of induction, only genome-sized RNA (35S, 7.8 kilobases) was present in augmented amounts, whereas the major subgenomic RNA (24S, 3,8 kilobases) did not appear until at least 30 to 60 min postinduction. The sequential appearance of these RNAs, the probable mRNA's for the gag and env proteins, paralleled the order of appearance of the gag and env proteins, respectively, after hormone treatment. An additional species of viral RNA (20S, 2.5 kilobases) was detected during these induction experiments, but the role of this RNA is not known. Both subgenomic RNAs contain sequences derived from both the 5' and 3' termini of genomic RNA and are presumably spliced. After dexamethasone induction of infected XC cells, we detected two smaller env-related proteins which were not found in full hormone induction. The functional role of these smaller proteins is not known. A previously reported smaller species of RNA (13S, 1.0 kilobase) did not appear to be induced and was shown to be cellular rather than viral in origin. In the fully induced infected XC and GR mammary tumor cells, the only viral RNAs present were the 35S and 24S RNAs. In addition, mammary tumors contained only these two viral RNAs. Thus, tumor cells appear to contain only the viral RNAs which direct the synthesis of the gag, pol, and env proteins of the virion. (Author abstract) (43 Refs)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

9. SPECIFIC INTEGRATION OF REV PROVIRUSES IN AVIAN BURSAL LYMPHOMAS.

Noori-Daloii MR, Swift RA, Kung HJ, Crittenden LB, Witter RL (c/o Kung) Dept. Biochemistry, Michigan State Univ., East Lansing, MI, 48824
Nature; 294(5841):574-576 1981

The structure of non-defective reticuloendotheliosis virus (nd Rev) proviruses in bursal lymphomas was characterized. The DNAs extracted from bursal tumors induced by the chick syncytial virus (CSV) strain of nd Rev were digested with restriction endonucleases, electrophoresed in agarose gels, and hybridized to probes specific for the nd Rev genomes and the oncogene of MC-29 virus. Linkage between REV proviruses and the c-myc gene was determined by EcoRI endonuclease digestion and confirmed by digestion with other restriction enzymes. The results suggested that in each tumor, at least one REV provirus was integrated next to the c-myc gene (on 1/2 homologous chromosomes); the latter gene was strongly implicated in lymphomagenesis. The REV proviruses in 22/25 tumors characterized were linked to c-myc. The sizes of the EcoRI linkage fragments varied from 11 to 22 kilobases (kb). The majority of viral sequences appeared to be present in the proviruses. Thus, structural alterations giving rise to the shortened 11-kb tumor-specific (TS) band probably involved the surrounding cellular sequence. The high intensity of the 11-kb TS band, along with its larger size compared to the unintegrated viral DNA, suggested that amplification of REV provirus and of its surrounding cellular sequences had occurred. Tumors 1 and 8 contained eight and one copies of the proviral sequences, respectively. Structural alteration in the REV provirus and its adjacent cellular sequences are discussed in relation to transformation. (23 Refs)

10. INABILITY OF AVIAN MYELOBLASTOSIS VIRUS RNA-DIRECTED DNA POLYMERASE TO TRANSCRIBE POLY(RU) DUE TO INSTABILITY OF THE OLIGO(DA):POLY(RU) COMPLEX.

Weiss GB, Carr BK
Div. Hematology-Oncology, Dept. Internal Medicine, Univ. Texas Medical Branch, G alveston, TX, 77550
Biochem Biophys Res Commun; 103(3):893-897 1981

Avian myeloblastosis virus DNA polymerase is unable to transcribe poly(rU). The problem with oligo(dA) as primer is in initiation of DNA synthesis. This finding, together with the observation that (dA:rU) duplexes are unstable, suggests that it is the instability of the primer:template complex which causes the lack of transcription of poly(rU). (11 Refs)

11. NUCLEOTIDE SEQUENCE OF THE GENOME OF A MURINE SARCOMA VIRUS.

Van Beveren C, Van Straaten F, Galleshaw JA, Verma IM
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
Cell; 27(1,part2):97-108 1981

The complete nucleotide sequence of a molecularly cloned, biologically infectious mouse sarcoma virus DNA has been determined. It is 5,833 nucleotides in length. It contains two long terminal repeats, the coding capacity for the entire gag gene, portions of the pol gene and the entire transforming gene. Synthesis of the 538 amino acid-long gag gene-encoded polypro-

tein Pr62gag initiates 596 nucleotides downstream from the 5'-cap nucleotide. The cleavage of gag polyprotein to mature core proteins, NH2-p15-p12-p30-p10-COOH, does not involve the loss of any amino acids. The complete nucleotide sequence of the cellular homolog (c-mosMo) of viral transforming gene v-mosMo has been determined. There are 25 single-base changes in an uninterrupted sequence of 1,157 nucleotides. Of these, 21 changes are in the coding region and they alter only 11 amino acids with respect to v-mosMo gene product. The homologous and nonhomologous regions between mouse sarcoma viral DNA and its progenitor, murine leukemia virus DNA, have been identified. Most deletions appear to have occurred by homologous recombination. A sequence GACC or its inverse GGTC is present in the vicinity of all substitutions and deletions. The codon usage of the gag gene is quite different from that of the c-mosMo gene. (Author abstract) (73 Refs)

12. STRUCTURE OF THE BABOON ENDOGENOUS VIRUS GENOME: NUCLEOTIDE SEQUENCES OF THE LONG TERMINAL REPEAT.

Tamura T, Noda M, Takano T
Dept. Microbiology, Keio Univ. Sch. Medicine, Shinjuku-ku, Tokyo, Japan
Nucleic Acids Res; 9(23):6615-6626 1981

The entire nucleotide sequence of the long terminal repeat (LTR) of baboon endogenous virus (BaEV) M7 was determined, which consisted of 554 base pairs (bp). At both ends of the LTR, 13 bp sequences, AAATGAAAAGTAA and TGATTCTAACATC, were detected to be inverted repeats. The structure with these inverted repeats resembles those of other retroviruses and transposable elements. A Hogness box, TATAAAA, and a putative poly(A)-addition signal, AGTAAA, were present within the right-hand half of the LTR, where the initiation and termination of the viral RNA synthesis seems to occur in the integrated BaEV gen ome. The primer-binding site of at least 14 bp long was found just outside of the LTR where the strong stop DNA started, and the primer for reverse transcription in BaEV seemed to be tRNA-Pro. Several structural features are commonly detected in the LTRs of BaEV and other retroviruses. Our studies suggest that BaEV has evolved from a common ancestor with other mammalian Type C viruses. Close relationships between BaEV and a feline endogenous virus, RD114, are demonstrated. (Author abstract) (33 Refs)

13. STRUCTURE, VARIATION AND SYNTHESIS OF RETROVIRUS LONG TERMINAL REPEAT.

Temin HM
McArdle Lab., Univ. Wisconsin, Madison, WI, 53706
Cell; 27(1,part2):1-3 1981

Nucleotide sequence data are reported for the long terminal repeats (LTRs) of cloned avian leukosis and sarcoma viruses, Moloney murine leukemia and sarcoma viruses, spleen necrosis virus, and mouse mammary tumor virus. Mechanisms of LTR synthesis and of the role of the LTR in retrovirus life cycles are discussed. (9 Refs)

14. STRUCTURE, STABILITY, METHYLATION, EXPRESSION AND GLUCOCORTICOID INDUCTION OF ENDOGENOUS AND TRANSFECTED PROVIRAL GENES OF MOUSE MAMMARY TUMOR VIRUS IN MOUSE FIBROBLASTS.

Hynes NE, Rahmsdorf U, Kennedy N, Fabiani L, Michalides R, Nusse R, Groner B
Kernforschungszentrum Karlsruhe, Institut fur Genetik und fur Toxikologie, Postfach 3640, D-7500 Karlsruhe, W. Germany
Gene; 15(4):307-317 1981

A sequence of mouse genomic DNA containing an endogenous mouse mammary tumor virus (MMTV) provirus, which was isolated by molecular cloning in lambda vector phage, has been reintroduced into cultured mouse L cells, using the thymidine-kinase (TK) cotransfection technique. Transfection of mouse cells with a lambda-MMTV recombinant DNA molecule, MMTV RNA expressed in the GR-40-transfected L cells, stability of transfected MMTV DNA in LTK+GR-40+ cells, hypomethylation of MMTV-transfected DNA, and MMTV-specific proteins in LTK+GR-40+-tranfected cells are discussed. Individual cell clones that acquired the transfected MMTV proviral DNA have been isolated. The transfected DNA remains stable in these cells and does not become methylated. It is transcribed into MMTV RNA, and the transcription is stimulated by glucocorticoid hormones. Results suggested that the transfected TK DNA and the MMTV proviral DNA are linked. Clone GR-40 contains a region of DNA which mediates the response to glucocorticoid. (39 Refs)

15. GENETIC MAPPING OF ENDOGENOUS MOUSE MAMMARY TUMOR VIRUSES: LOCUS CHARACTERIZATION, SEGREGATION, AND CHROMOSOMAL DISTRIBUTION.

Traina VL, Taylor BA, Cohen JC
Dept. Microbiology and Immunology, Tulane Medical Center, New Orleans, LA, 70112
J Virol; 40(3):735-744 1981

The restriction endonuclease EcoRI has been used to study the inheritance of strain differences in endogenous mouse mammary tumor virus DNA sequences. This enzyme, which cleaves at only one site within the nondefective viral genome, generates DNA fragments containing mouse mammary tumor virus sequences which vary in size according to the locations of EcoRI restriction sites in the flanking mouse sequences, thereby defining unique integration sites of the viral genome. Recombinant inbred strains of mice have been used to study the inheritance of these DNA fragments which hybridize to mouse mammary tumor virus cDNA sequences. The results define 11 segregating units consisting of one or two fragments. These units were shown to segregate among the recombinant inbred strains, and in some instances linkage was established. Two units were shown to be linked on chromosome 1. Another unit was mapped to chromosome 7, which is presumably identical to the previously defined genetic locus Mtv-1. One other mouse mammary tumor virus locus was tentatively assigned to chromosome 6. The results are consistent with the view that integration of mouse mammary tumor virus can take place at numerous sites within the genome, and once inserted, these proviruses appear to be relatively stable genetic entities. (Author abstract) (45 Refs)

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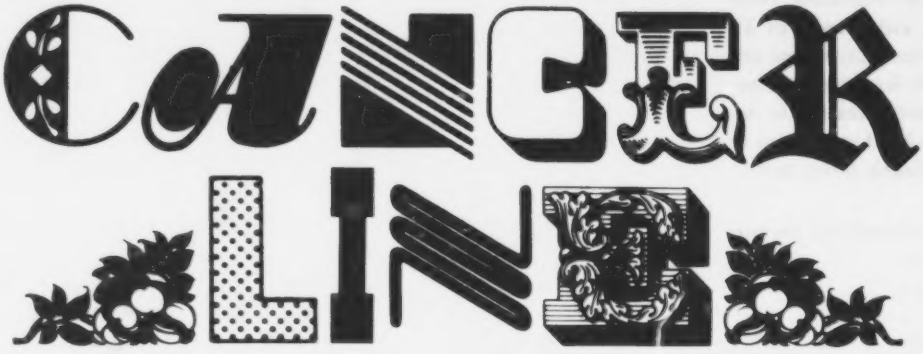

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RNA VIRUSES ASSOCIATED WITH CANCER

Molecular Biology

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VIRAL PROTEINS

1. IN VITRO PHOSPHORYLATION OF MURINE LEUKEMIA VIRUS PROTEINS: SPECIFIC PHOSPHORYLATION OF PR65GAG, THE PRECURSOR IN THE INTERNAL CORE ANTIGENS.

Yoshinaka Y, Luftig RB
(c/o Luftig) Dept. Microbiology and Immunology, Univ. South Carolina Sch. Medicine, Columbia, SC, 29208
Virology; 116(1):181-195 1982

In vitro phosphorylation of Nonidet P-40 disrupted murine leukemia virus with [gamma-32P]ATP showed that Pr65gag, which is present as a relatively minor component (14% of total Coomassie blue-stained virus proteins), was the major (80% labeling) phosphorylated species. A small amount of 32P label (ie, 3-9%) was associated with proteins whose mobility on sodium dodecyl sulfate-polyacrylamide gel electrophoresis was the same as Pr40gag, p12, and p10. In contrast, long-term (20 hr) in vivo phosphorylation with [32P]orthophosphate showed that p12 was the most highly phosphorylated viral protein species. In neither case did we find any label associated with the major virion proteins, p30, p15, or gp70. These results suggest

HE 20.3173/2:

that phosphorylation is specific for certain viral proteins and, further, that if Pr65gag is packaged as an uncleaved polyproteins into virions it remains relatively unphosphorylated. We have also found that the kinase activity which phosphorylates Pr65gag in vitro is tightly associated with immature core subparticles and can phosphorylate the exogenous substrate phosvitin. The same or an additional kinase activity can also be detected in the soluble fraction of detergent-disrupted virus using endogenous viral proteins or phosvitin as substrate. Based on an analysis of the tryptic peptide patterns of pPr65gag phosphorylated in vitro and in vivo, it appears that the in vitro phosphorylation of Pr65gag occurs at serine residues which are identical to or similar to those found for pPr65gag in vivo, ie, on pp12, as well as at another site. This other site is also probably on p12 since in vitro phosphorylated pPr65gag, when cleaved in vitro by the murine Pr65gag proteolytic factor, produces as its initial product a phosphorylated band that has the mol wt of pPr27gag and the antigenic determinants of p15 and p12. (Author abstract) (24 Refs)

2. ENV GENE PRODUCTS OF AKR DUAL-TROPIC VIRUSES: EXAMINATION OF PEPTIDE MAPS AND CELL SURFACE EXPRESSION.

Famulari NG, English KJ
Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
J Virol; 40(3):971-976 1981

The env gene products of nine AKR dual-tropic murine leukemia viruses were compared by peptide mapping and were assayed for expression on the cell surface of infected fibroblasts. Seven virus isolates expressed the env gene polyprotein on the cell surface. The env gene products of six of these seven viruses had identical peptide maps. The analysis of structure and expression of env gene products carried out in this study characterizes a subset of dual-tropic murine leukemia viruses shown by others to be thymotropic. (Author abstract) (21 Refs)

3. STRUCTURAL DOMAINS OF ENDOGENOUS MURINE LEUKEMIA VIRUS GP70S CONTAINING SPECIFIC ANTIGENIC DETERMINANTS DEFINED BY MONOCLONAL ANTIBODIES.

Pinter A, Honnen WJ, Tung JS, O'Donnell PV, Hammerling U
Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
Virology; 116(2):499-516 1982

Eight distinct antigenic determinants, or epitopes (labeled a-h) were identified on endogenous ecotropic murine leukemia virus (MuLV) gp70s using a series of murine and rat monoclonal antibodies. These epitopes were characterized by their distribution patterns in a panel of cloned MuLVs, and by their localization in fragments of gp70 generated either by spontaneous breakdown of purified gp70, or by controlled proteolysis of gp70 in solubilized virions. A major 34K carboxy terminal fragment was formed wh ich contained epitopes b, c, and f. This fragment also possessed the p15(E) disulfide linkage site, and contained approximately four complex (Type I) carbohydrate chains. An amino terminal 35K fragment contained epitopes a, d, g, and h, and possessed two glycosylated sites, including a site which occasionally retained an endoglycosidase H-sensitive oligosaccharide chain. A related 49K fragment was also obtained which included the entire 35K region and contained an additional sequence bearing epitope e. In a series of dualtropic MCF-type viruses studied, only those epitopes located in the 32K fragment were ever retained, indicating that for these recombinant viruses at least a portion of that domain was derived from the ecotropic parent. A model is presented indicating the likely orientation of these fragments and their structural characteristics. (Author abstract) (38 Refs)

4. LETHAL EFFECT OF THE ABELSON MURINE LEUKEMIA VIRUS TRANSFORMING GENE PRODUCT.

Ziegler SF, Whitlock CA, Goff SP, Gifford A, Witte ON
Molecular Biology Inst., Dept. Microbiology, Univ. California Los Angeles, Los Angeles, CA, 90024
Cell; 27(3):477-486 1981

Infection of NIH/3T3 and Balb/c/3T3 fibroblastic cell lines by Abelson virus results in stable transformation in most cases. We have observed that certain rodent cell lines, including some Balb/c/3T3 sublines, do not maintain stable transformation following infection. Within 3 to 4 wk of infection of these cell lines a large fraction of the cells is not recoverable upon serial passage and appear to be killed. The residual population of cells displays a flat, nontransformed phenotype. Concomitant with this reversion is the loss of the integrated provirus and loss of expression of the Abelson virus transforming protein. This loss of the integrated provirus is specific to transformation-competent viral strains. The effect of Abelson virus on these cells appears to be, in part, due to a lethal effect of the transforming protein on the cells. We have selectively isolated mutants of Abelson virus that stably transformed these rodent cell lines without expressing a lethal effect. Analysis of the nucleic acid and protein structure of these mutant virus strains showed that the transforming proteins are shorter, having lost expression of the carboxy-terminal one-third of the protein. We suspect that the carboxy-terminal portion of the wild-type Abelson protein may modulate the in vivo expression of the protein kinase activity and that this may cause the transforming protein to have a deleterious effect on certain cell lines. (Author abstract) (33 Refs)

5. EVIDENCE THAT PP60SRC, THE PRODUCT OF THE ROUS SARCOMA VIRUS SRC GENE, UNDERGOES AUTOPHOSPHORYLATION.

Purchio AF
Molecular Genetics, Inc., Minnetonka, MN, 55343
J Virol; 41(1):1-7 1982

The product of the Rous sarcoma virus src gene, pp60src, was purified greater than 100,000-fold by a combination of ion-exchange and immunoaffinity chromatography. Incubation of pp60src purified in this fashion with [32P-gamma]ATP resulted in a single 32P-labeled protein when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Staining of these gels with silver nitrate showed a predominant 60,000-dalton polypeptide which comigrated with the protein labeled with 32P in vitro. Partial digestion of this protein with V8 protease after in vitro iodination indicated that it was pp60src. These results suggest that pp60src is able to autophosphorylate. (Author abstract) (21 Refs)

6. SYNTHETIC PEPTIDE FRAGMENT OF SRC GENE PRODUCT INHIBITS THE SRC PROTEIN KINASE AND CROSSREACTS IMMUNOLOGICALLY WITH AVIAN ONC KINASES AND CELLULAR PHOSPHOPROTEINS.

Wong TW, Goldberg AR
Rockefeller Univ., New York, NY, 10021
Proc Natl Acad Sci USA; 78(12):7412-7416 1981

All the known avian sarcoma viruses have associated protein kinase activities that phosphorylate tyrosine residues of their target proteins. A decapeptide fragment of pp60src of Rous sarcoma virus (RSV), residues 415-425, and an analog of that sequence have been chemically synthesized by solid-phase methods. The two decapeptides were not phosphorylated by

pp60src of RSV, P90 of Y73 avian sarcoma virus, or P140 of Fujinami sarcoma virus. However, both peptides were able to inhibit competitively the kinase activities associated with the transforming proteins. Antiserum was raised against one of the peptides and IgG was purified from the serum by affinity chromatography. The antibody was able to precipitate pp60src of RSV as well as P90 of Y73 virus from cells infected with these viruses. The antibody also precipitated a number of high molecular weight phosphoproteins from normal chicken and rat fibroblasts and from several lines of virus-transformed cells. (Author abstract) (29 Refs)

7. A TS T MUTANT OF SCHMIDT RUPPIN STRAIN OF ROUS SARCOMA VIRUS RESTRICTED AT 39.5 C FOR THE MORPHOLOGICAL TRANSFORMATION AND THE TUMORIGENICITY OF CHICKEN EMBRYO FIBROBLASTS.

Poirier F, Lawrence D, Vigier P, Jullien P
Institut Curie Section Biologie, Centre Universitaire, 91405 Orsay, France
Int J Cancer; 29(1):69-76 1982

In order to investigate a possible correlation between in vitro transformation and tumorigenicity in ovo, a new temperature-sensitive class T mutant of Rous Sarcoma Virus was isolated with a lower (39.5 C) restrictive temperature for morphological transformation. This lower restrictive temperature was compatible with the survival of chicken and duck eggs for the tumorigenicity studies. In chicken embryo fibroblasts (CEF) infected by this new mutant, PA 17, and cultured at 39.5 C, increase of hexose u ptake, plasminogen activator production and anchorage-independent growth were only partially restricted, requiring incubation at 41.5 C for a complete shut-off. Tumorigenicity in chicken and duck eggs inoculated with CEF infected and transformed by PA 17 was restricted at 39.5 C, correlating well with the restriction of morphological transformation at this temperature. The kinase activity of the transforming protein pp60src in lysates of PA 17 infected cells cultured at permissive or restrictive temperatures was labile in RIPA buffer, as in the case of some previously examined ts T mutants. In the non-ionic detergent NP40 buffer, the kinase activity of PA 17 infected cell lysates was better conserved and showed a moderate temperature dependence. These results suggest that, in spite of the correlations between the transformed cell phenotype in vitro and cell tumorigenicity in ovo, it is difficult to establish a quantitative relationship. (Author abstract) (39 Refs)

8. STRUCTURE AND PHOSPHORYLATION OF THE FUJINAMI SARCOMA VIRUS GENE PRODUCT.

Pawson T, Kung TH, Martin GS
Dept. Microbiology, Univ. British Columbia, Vancounver, British Columbia V6T 1W5, Canada
J Virol; 40(3):665-672 1981

The Fujinami avian sarcoma virus (FSV) transforming gene product, P140, is a fusion protein which contains both gag-related and FSV-specific methionine-containing tryptic peptides. The virion protease p15 cleaved P140 into two fragments: an N-terminal 33K fragment which contained all but one of the gag-related tryptic peptides and a C-terminal 120K fragment which contained all of the FSV-specific tryptic peptides. The 33K gag-related fragment from P140 phosphorylated in FSV-transformed cells contained only phosphoserine, whereas the 120K C-terminal FSV-specific fragment contained both phosphoserine and phosphotyrosine. P140 isolated from cells infected at the nonpermissive temperature with an isolate of FSV which is temperature sensitive for transformation had a normally phosphorylated 33K fragment, but a hypophosphorylated 120K fragment deficient in both phosphotyrosine and phosphoserine. When P140 was immunoprecipitated from cells and phosphorylated in vitro at tyrosine residues in the immune complex kinase reaction, only the FSV-specific fragment was labeled. These data define the structure of FSV P140 and locate the phosphorylated amino acids within the two regions of the polypeptide. (Author abstract) (15 Refs)

9. GENE PRODUCTS OF MCDONOUGH FELINE SARCOMA VIRUS HAVE AN IN VITRO-ASSOCIATED PROTEIN KINASE THAT PHOSPHORYLATES TYROSINE RESIDUES: LACK OF DETECTION OF THIS ENZYMATIC ACTIVITY IN VIVO.

Barbacid M, Lauver AV
Lab. Cellular and Molecular Biology, NCI, Bethesda, MD, 20205
J Virol; 40(3):812-821 1981

The primary translational product of the McDonough (SM) strain of feline sarcoma virus (FeSV) is a 180,000-dalton molecule, SM P180, that contains the p15-p12-p30 region of the FeLV gag gene-coded precursor protein and a sarcoma virus-specific polypeptide. In addition, cells transformed by SM-FeSV express a 120,000-dalton molecule, SM P120, that is highly related to the non-helper virus domain of SM P180. Both SM-FeSV gene products were found to be intimately associated with the membrane fraction of SM-FeSV-transformed cells. Immunoprecipitates containing SM P180 and SM P120 exhibited a protein kinase activity capable of phosphorylating tyrosine residues of both viral gene products but not immune immunoglobulin G molecules. By independently immunoprecipitating each of the two SM-FeSV proteins we found that most of the tyrosine-specific phosphorylating activity was associated with the SM P120 molecule. In vivo analysis of 32P-labeled SM P180 and SM P120 revealed their phosphoprotein nature; however, both molecules exhibited low levels of phosphorylation and did not contain phosphotyrosine residues. Finally, we did not detect any significant elevation in the levels of phosphotyrosine in the protein fraction of SM-FeSV transformants. Thus, if SM-FeSV were to induce malignant transformation by a mechanism involving phosphorylation of tyrosine residues, the viral gene products must interact with a small subset of cellular proteins that do not represent a significant fraction of the total cellular protein content. (Author abstract) (44 Refs)

10. ONCOGENES. A UNITARY CAUSE OF CANCER?

Erikson RL
4200 E Ninth Ave., Denver, CO, 80262
JAMA; 247(2):178-181 1982

The sarc gene and the sarc gene product of Rous sarcoma virus, relevance of tumor viruses to human cancer, number of transforming genes in RNA viruses, and usefulness of retroviruses in cancer research are discussed. The sarc gene induces fibrosarcomas in susceptible animals and causes malignant transformation of cultured fibroblasts. Its product is a 60,000-dalton phosphoprotein that is concentrated in plasma membranes of transformed cells; the protein has protein kinase activity and phosphorylates tyrosine residues in immunoglobulins and cellular proteins. Through genetic recombination, RNA tumor viruses have acquired host-cell genes in addition to the sarc gene. Following viral reinfection, the cellular genes are capable of transforming a second host cell into a cancer cell. Although sarc and cellular genes initiate the transformation process

through overproduction of gene products, a gene-specific substrate may be necessary to achieve transformation. A schematic diagram shows the malignant transformation of a normal cell after infection with avian sarcoma virus. (no Refs)

11. ONCOGENESIS BY RNA TUMOR VIRUSES.

Koyama T
Dept. Carcinostasis Res., Tokyo Univ. Medical Res. Inst., 4-6-1 Kindai, Minato-k u, Tokyo 108, Japan
Tanpakushitsu Kakusan Koso; 26(14):2121-2129 1981

The origin of retrovirus transforming genes and their oncogenic mechanisms were discussed with respect to literature data. Existence of oncogenic genes in lymphogenous leukemia virus, sarcoma viruses, and acute leukemia viruses was discussed. Oncogenic mechanisms of sarcoma viruses, acute leukemia viruses, and lymphogenous leukemia viruses were reviewed with the aid of analysis at the nucleic acid or protein levels. Various research showed that oncogenesis by retroviruses was not only due to promoter insertion; changes in the manifestation of the c-oncogenic gene, by introduction of DNA rearrangement methods, were also thought to be possible. (77 Refs) (In JPN)

12. ROUS SARCOMA VIRUS-INDUCED PHOSPHORYLATION OF A 50,000-MOLECULAR WEIGHT CELLULAR PROTEIN.

Brugge JS, Darrow D
Dept. Microbiology, State Univ. New York at Stony Brook, Stony Brook, NY, 11794
Nature; 295(5846):250-253 1982

Studies were performed to determine whether the 50,000-mol wt phosphotyrosine-containing protein pp50 is present in uninfected cells and whether phosphorylation of pp50 at tyrosine depends on Rous sarcoma virus (RSV)-induced transformation. Using serum from tumor-bearing rabbits (TBR), pp50 was immunoprecipitated from 32P-labeled chicken cells transformed by the SR-A strain of RSV. Comparison of the two-dimensional gel patterns of uninfected cell phosphoproteins with a mixture of the TBR immunoprecipitate and uninfected cell phosphoproteins indicated that the pp50 which is bound to the single virus-encoded polypeptide pp60src and precipitated RSV-transformed cells migrate just to the acidic side of pp50 present in uninfected cells. Results indicate that the phosphoserine-containing form of pp50 is present in uninfected chicken cells; on transformation by RSV, a second form of pp50 detectable by two-dimensional analysis of 32P-labeled proteins contains phosphotyrosine as well as phosphoserine. The phosphorylation of pp50 at tyrosine is not temperature-sensitive (TS) in cells infected with mutant viruses containing a TS defect in the src gene. The temperature stability might result from the strong interaction between the mutant pp60src protein and pp50. (25 Refs)

VIRAL RNA

13. CELLULAR GENES ANALOGOUS TO RETROVIRAL ONC GENES ARE TRANSCRIBED IN HUMAN TUMOUR CELLS.

Eva A, Robbins KC, Andersen PR, Srinivasan A, Tronick SR, Reddy P, Ellmore NW, Galen AT, Lautenberger JA, Papas TS, Westin EH, Wong-Staal F, Gallo RC, Aaronson SA
Lab. Cellular and Molecular Biology, NCI, NIH, Bethesda, MD, 20205
Nature; 295(5845):116-119 1982

Polyadenylated RNAs of certain human tumor cell lines are shown to contain transcripts related to the cell-derived transforming onc genes of molecularly cloned primate, murine or avi an transforming retrovirus genomes. Thus, analogues of retroviral transforming genes are both present and frequently expressed in human neoplastic cells. (Author abstract) (52 Refs)

14. NUCLEOTIDE SEQUENCE OF THE 5' NONCODING REGION AND PART OF THE GAG GENE OF ROUS SARCOMA VIRUS.

Swanstrom R, Varmus HE, Bishop JM
Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143
J Virol; 41(2):535-541 1982

Several functions of the retrovirus genome involve structural features in the vicinity of its 5' terminus. In an effort to further elucidate the relationship between structure and function in retrovirus RNA, we have determined the sequence of the first 1,010 nucleotides at the 5' end of the genome of Rous sarcoma virus by using the Maxam-Gilbert method to sequence suitable domains in cloned Rous sarcoma virus DNA. The results (i) locate the initiation codon for the gag gene of Rous sarcoma virus 372 nucleotides from the 5' end of viral RNA; (ii) demonstrate that this codon is preceded by three methionine codons that are apparently not used in translation; (iii) sustain previous conclusions that the principal site to which ribosomes bind on the Rous sarcoma virus genome in vitro does not contain the initiation codon for gag; (iv) permit deduction of the amino acid sequence of a viral structural protein, p19; (v) confirm the amino-terminal sequence of Pr76gag; and (vi) substantiate the identification of a splice donor site described in the accompanying manuscript. (Author abstract) (46 Refs)

15. THE LEADER SEQUENCE OF THE SUBGENOMIC MRNA'S OF ROUS SARCOMA VIRUS IS APPROXIMATELY 390 NUCLEOTIDES.

Hackett PB, Swanstrom R, Varmus HE, Bishop JM
Dept. Genetics and Cell Biology, Univ. Minnesota, St. Paul, MN, 55108
J Virol; 41(2):527-534 1982

The subgenomic mRNA's of Rous sarcoma virus share a common 5' leader sequence spliced from genomic RNA. We have examined the 5' terminal sequences of four Rous sarcoma virus RNAs: virion RNA and three species of intracellular mRNA which direct the synthesis of the RSV gene products. The lengths of the leaders on the RNAs were determined by the extent that they could protect cloned Rous sarcoma virus DNA fragments from S1 nuclease digestion after RNA-DNA hybridization. We found that the subgenomic mRNA's that direct the synthesis of the env and src gene products have uninterrupted spliced leader sequences, of approx 390 nucleotides, whereas virion RNA and full-length intracellular viral RNA

have 5' termini homologous to the cloned viral DNA probe over at least the first 735 bases. In the accompanying manuscript we have determined the nucleotide sequence of the 5' end of the Rous sarcoma virus genome, including the candidate splice donor site identified here. (Author abstract) (46 Refs)

16. A TEMPERATURE-SENSITIVE LESION AFFECTING LEVELS OF TRANSFORMATION-SPECIFIC VIRAL RNA IN A MUTANT OF AVIAN SARCOMA VIRUS PRCII.

Hirano A, Wong TC, Vogt PK
(c/o Vogt) Dept. Microbiology, Univ. Southern California, Sch. Medicine, 2011 Zonal Ave., Los Angeles, CA, 90033
Virology; 116(2):646-649 1982

LA46 is a mutant of avian sarcoma virus PRCII that is temperature sensitive (ts) in the maintenance of oncogenic transformation and in the production of infectious virus. These ts properties are independent of the helper virus. At the nonpermissive temperature LA46-infected cells also fail to produce significant quantities of the transformation-specific protein, and the amount of intracellular transformation-specific viral RNA is reduced at least 100-fold. The probable primary defect of LA46, a temperature sensitivity in transformation-specific viral RNA, can account for the phenotype of the mutant. The molecular mechanism of this defect is currently investigated. (Author abstract) (7 Refs)

17. ASSOCIATION OF 3' TERMINAL RNA SEQUENCES WITH AVIAN LEUKOSIS VIRUSES CAUSING A HIGH INCIDENCE OF OSTEOPETROSIS.

Schmidt EV, Kenne JD, Linial M, Smith RE
(c/o Smith) Dept. Microbiology and Immunology, Duke Univ. Medical Center, Durham, NC, 27710
Virology; 116(1):163-180 1982

The T1 ribonuclease fingerprints of the genomic RNAs of four osteopetrosis-inducing, avian leukosis viruses were compared with fingerprints of the RNAs from four unrelated avian retroviruses. Two oligonucleotides at the 3' end of the genomes of all osteopetrosis-inducing viruses were not present in the RNAs of the four viruses that did not induce osteopetrosis. This evidence suggests that genetic information related to the induction of a high frequency of osteopetrosis is present at the 3' end of the genomes of these viruses. (Author abstract) (41 Refs)

18. STRUCTURE OF RETROVIRAL RNAS PRODUCED BY CELL LINES DERIVED FROM SPONTANEOUS LYMPHOMAS OF AKR MICE.

Pedersen FS, Crowther RL, Hays EF, Nowinski RC, Haseltine WA
Dept. Molecular Biology, Univ. Aarhus, DK-8000 Aarhus C, Denmark
J Virol; 41(1):18-29 1982

The retrovirus expression of eight independent lymphoid cell lines derived from spontaneous thymomas of AKR mice was investigated. The RNase T1 finger-prints of viral 70S RNA produced by these cell lines were compared with genome structures of the non-leukemogenic Akv virus and with two types of cloned leukemogenic viruses derived from one of the thymoma cell lines. Viral RNAs from three cell lines, SL3, 4, and 7, were indistinguishable from one another. The fingerprint patterns indicated that these cell lines produce equal amounts of two prototype, leukemogenic SL viruses that were previously isolated from the SL3 cell line. Viral RNA produced by the SL1 and SL2 cell lines contained similar components, but at a different ratio. Two other cell lines (SL5 and SL11) produced viral RNAs that resemble those of AKR mink ce ll focus-forming viruses. One additional line, SL9, produced viral RNA of a novel structure. The complex pattern of viral RNA expression observed for these lymphoid cell lines can be interpreted in terms of recombination among three types of endogenous viral sequences: the Akv virus, a xenotropic virus, and an SL (for spontaneous leukemia) virus. (Author abstract) (25 Refs)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

19. MCDONOUGH FELINE SARCOMA VIRUS: CHARACTERIZATION OF THE MOLECULARLY CLONED PROVIRUS AND ITS FELINE ONCOGENE (V-FMS).

Donner L, Fedele LA, Garon CF, Anderson SJ, Sherr CJ
(c/o Sherr) Viral Pathology Section, NCI, Bethesda, MD, 20205
J Virol; 41(2):489-500 1982

The genetic structure of the McDonough strain of feline sarcoma virus (SM-FeSV) was deduced by analysis of molecularly cloned, transforming proviral DNA. The 8.2-kilobase pair SM-FeSV provirus is longer than those of other feline sarcoma viruses and contains a transforming gene (v-fms) flanked by sequences derived from feline leukemia virus. The order of genes with respect to viral RNA is 5'-gag-fms-env-3', in which the entire feline leukemia virus env gene and an almost complete gag sequence are represented. The complexity of the v-fms gene (3.1 +-0.3 kilobase pairs) is approximately two-fold greater than the viral oncogene sequences (v-fes) of Snyder-Theilen and Gardner-Arnstein FeSV. By heteroduplex, restriction enzyme, and nucleic acid hybridization analyses, v-fms and v-fes sequences showed no detectable homology to one another. Radiolabeled DNA fragments representing portions of the two viral oncogenes hybridized to different EcoRI and HindIII fragments of normal cat cellular DNA. Cellular sequences related to v-fms (designated c-fms) were much more complex than c-fes and were distributed segmentally over more than 40 kilobase pairs in cat DNA. Comparative structural studies of the molecularly cloned proviruses of Snyder-Theilen, Gardner-Arnstein, and SM-FeSV showed that a region of the feline leukemia virus genome derived from the pol-env junction is represented adjacent to v-onc sequences in each FeSV strain and may have provided sequences preferred for recombination with cellular genes. (Author abstract) (55 Refs)

20. CHARACTERIZATION OF RETICULOENDOTHELIOSIS VIRUS STRAIN T DNA AND ISOLATION OF A NOVEL VARIANT OF RETICULOENDOTHELIOSIS VIRUS STRAIN T BY MOLECULAR CLONING.

Chen IS, Mak TW, O'Rear JJ, Temin HM
McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
J Virol; 40(3):800-811 1981

Reticuloendotheliosis virus strain T (REV-T) is a highly oncogenic avian retrovirus which causes a rapid neoplastic disease of the lymphoreticular system. Upon infection, this virus gives rise to two species of unintegrated linear viral DNA, which are 8.3 and 5.5 kilobase pairs long and rep resent the helper virus (REV-A) and the oncogenic component (REV-T), respectively. Restriction endonuclease cleavage maps of these two DNA components indicate that REV-T DNA has a large

portion of the genome deleted with respect to REV-A DNA and a substitution about 0.8 to 1.5 kilobase pairs long that is unrelated to REV-A DNA. These additional sequences comprise the putative transforming region of REV-T (rel). A chicken spleen cell line transformed by REV-T produced virus which upon infection gives rise to three species of unintegrated linear viral DNA (8.3, 5.5, and 3.3 kilobase pairs). We isolated the proviruses of the 8.3-and 3.3-kilobase pair species from this cell line by cloning in the phage vector Charon 4A. Restriction enzyme mapping showed that the two proviral clones are proviruses of REV-A and a variant of REV-T, respectively. A subclone of the variant REV-T provirus specific for the rel sequences of REV-T was used as a hybridization probe to demonstrate that the rel sequences are different from the putative transforming sequences of Schmidt-Ruppin Rous sarcoma virus strain A, avian myelocytomatosis virus, avian myeloblastosis virus, avian erythroblastosis virus, Abelson murine leukemia virus, and Friend erythroleukemia virus. In addition, the rel-specific hybridization probe was used to identify a specific set of sequences which are present in uninfected avian DNAs digested with several restriction enzymes. The corresponding cell sequences are not arranged like rel in REV-T. (Author abstract) (46 Refs)

21. CLONING AND CHARACTERIZATION OF AN ENVELOPE-SPECIFIC PROBE FROM XENOTROPIC MURINE LEUKEMIA PROVIRAL DNA.

Buckler CE, Hooggan MD, Chan HW, Sears JF, Khan AS, Moore JL, Hartley JW, Rowe WP, Martin MA
Lab. Viral Diseases, Natl. Inst. Allergy and Infectious Diseases, Bethesda, MD, 20205
J Virol; 41(1):228-236 1982

An 8.9-kilobase EcoRI restriction fragment was cloned from mink cells chronically infected with NFS-Th-1 xenotropic murine leukemia virus by using a lambda phage host vector system. After its transfer into pBR322, the EcoRI DNA insert was characterized and found to contain 6.7 kilobases of proviral DNA sequences and 2.2 kilobases of mink cellular DNA flanking the 5' end of the viral genome. A 500-base pair fragment which was located at the 3' terminus of the cloned DNA insert and which mapped to the env region of xenotropic proviral DNA was subcloned into pBR322. This xenotropic envelope proviral DNA segment did not hybridize to ecotropic murine leukemia proviruses but did anneal to representative alpha and beta xenotropic and seven different mink cell focus-inducing proviral DNAs. The cloned xenotropic envelope-specific probe was also used in blot hybridization experiments to analyze the arrangement of related sequences in preparations of different mouse liver DNAs. (Author abstract) (34 Refs)

22. MAPPING OF NONCONDITIONAL AND CONDITIONAL MUTANTS IN THE SRC GENE OF PRAGUE STRAIN ROUS SARCOMA VIRUS.

Fincham VJ, Chiswell DJ, Wyke JA
(c/o Wyke), Imperial Cancer Res. Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, England
Virology; 116(1):72-83 1982

Restriction endonuclease EcoRI digestion of the viral DNA of 12 nonconditional transformation defective (td) mutants of Prague strain Rous sarcoma virus (PR-RSV) has divided these mutants into two groups. Five mutants possess an EcoRI B (src gene-containing) fragment of the same size as that from wild type PR-RSV and thus these mutants have no detectable diminution in the transforming src gene. The other 7 mutants bear deletions of 1.0 to 1.8 kilobases in the 3.2-kilobase EcoRI B fragment. The extents of these deletions have been mapped using a number of restriction endonucleases and by comparing these results with studies on the nucleotide sequence of src we conclude that the td mutants have deleted sequences at the 5' end of src, and in some cases also in regions between src and env, leaving intact at least some 3' src sequences. These td mutants recombine in differing patterns with 14 temperature-sensitive (ts) src gene mutants. This enables many of the ts mutations to be localized in limited regions of src, 10 of them being clustered in the 3' 40% of the gene the remaining four bearing at least one mutation in the 5' 60% of src. A nonconditional src gene mutant that transforms cells to a fusiform as opposed to round cell morphology (td SF/LO 104) also possesses a lesion that maps in the 5' 60% of the src gene. (Author abstract) (28 Refs)

23. COMPARISON OF THE RESTRICTION ENDONUCLEASE MAPS OF UNINTEGRATED PROVIRAL DNAS FROM FOUR XENOTROPIC MURINE LEUKEMIA VIRUSES.

Joseph DR
Robert V. Slater Lab., Papanicolaou Cancer Res. Inst., Miami, FL, 33136
J Virol; 40(3):963-970 1981

From purified linear and superhelical DNAs, the restriction endonuclease maps of four xenotropic murine leukemia virus DNAs from NFS, NZB, BALB/c, and AKR mice were determined with 10 restriction endonucleases. Each xenotropic proviral DNA was found to have a unique restriction endonuclease map, with differences in the gag, pol, env, and terminal repeated sequence regions. However, type-specific SacI and EcoRI sites in the env region were identical in all four xenotropic murine leukemia virus DNAs and were not found in ecotropic murine leukemia virus DNA. Comparison of the xenotropic murine leukemia virus DNA maps with maps of ecotropic murine leukemia virus DNA showed that the pol and terminal repeated sequence regions were highly conserved. Other similarities in ecotropic and some xenotropic viral DNAs suggest common origins. (Author abstract) (34 Refs)

24. MULTIPLE ARRANGEMENTS OF VIRAL DNA AND AN ACTIVATED HOST ONCOGENE IN BURSAL LYMP HOMAS.

Payne GS, Bishop JM, Varmus HE
Dept. Biochchemistry and Biophysics, Univ. California, San Francisco, CA, 94143
Nature; 295(5846):209-214 1982

Proviruses of avian leukosis virus (ALV) are located in the vicinity of a putative cellular oncogene (c-myc) in ALV-induced bursal lymphomas. Enhanced expression of c-myc occurs in association with proviruses found in any of three configurations: (I) on the 5' side ('upstream') of c-myc in the same transcriptional orientation; (II) on the 3' side '(downstream') of c-myc in the same orientation; (III) upstream, in the transcriptional orientation opposite to that of c-myc. Thus, activation of adjacent cellular genes by retroviral DNA can involve mechanisms other than provision of a transcriptional promoter. (Author abstract) (38 Refs)

25. ALTERED METHYLATION OF ENDOGENOUS VIRAL PROMOTER SEQUENCES DURING MAMMARY CARCINOGENESIS.

Breznik T, Cohen JC
Dept. Microbiology and Immunology, Tulane Univ., Sch. Medicine, New Orleans, LA, 70112
Nature; 295(5846):255-257 1982

The methylation pattern of mouse mammary tumor virus (MMTV) was examined. Origin of the 2.1-and 1.4-kilobase (kb) HpaII fragments was determined by comparing MspI digests from various inbred mouse strains. Comparison of MspI digestion of DNA from the CBA/CaJ mouse strain (which includes units II and III) with that obtained with DNA from BALB/c (which includes units I, II, and III), indicated that the 2.1-and 1.4-kb MspI-HpaII fragments are derived from the subgenomic unit I. HpaII digestion of DNA from BALB/c mammary tumor 1247 produced several virus-specific fragments in addition to those derived from unit I, indicating that demethylation of other endogenous proviruses has occurred in this tumor. The origin of additional sites of demethylation in tumor 1247 was identified by EcoRI-HpaII double digestions. Demethylation of proviral units during tumorigenesis could be ascertained by determining which EcoRI bands are sensitive to further cleavage by HpaII. Results showed that the methylation pattern of endogenous virus sequences is specifically altered during tumorigenesis in uninfected mice. In addition to transformation by promoter insertion which may occur during exogenous MMTV infection, carcinogenesis by promoter derepression may occur; such an event is suggested by the observed demethylation of either the defective MMTV sequence (unit I) or specifically the 3' half of the nondefective proviruses (units II and III). (20 Refs)

26. AVIAN MYELOBLASTOSIS VIRUS TRANSFORMING GENE IS RELATED TO UNIQUE CHICKEN DNA REGIONS SEPARATED BY AT LEAST ONE INTERVENING SEQUENCE.

Perbal B, Baluda MA
(c/o Baluda), Univ. California Los Angeles Sch. Medicine, Los Angeles, CA, 90024
J Virol; 41(1):250-257 1982

Identification of several additional restriction endonuclease sites within the c ellular substitution (amv) inserted into the avian myeloblastosis virus proviral genome has permitted us to isolate different regions of the amv sequence. These subsets of the avian myeloblastosis virus transforming gene have been cloned in the plasmid pBR322 and used as hybridization probes to investigate the topology of homologous (proto-amv) normal chicken DNA sequences. The results showed that the cellular proto-amv sequences in C/O chicken DNA are interrupted by at least one intervening sequence. A partial arrangement of the proto-amv sequences is presented. (Author abstract) (32 Refs)

27. CELLULAR MOLONEY MURINE SARCOMA (C-MOS) SEQUENCES ARE HYPERMETHYLATED AND TRANSCRIPTIONALLY SILENT IN NORMAL AND TRANSFORMED RODENT CELLS.

Gattoni S, Kirschmeier P, Weinstein IB, Escobedo J, Dina D
Div. Environmental Sciences, Inst. Cancer Res., Columbia Univ., New York, NY, 10032
Mol Cell Biol; 2(1):42-51 1982

The extent of methylation of the oncogenic v-mos sequence of Moloney murine sarcoma virus and its homologue present in normal cells of various vertebrate species (c-mos) was investigated. Results indicated that normal development and differentiation, prolonged serial passage, or transformation (either spontaneous or induced by radiation, chemical carcinogens, or polyoma virus) are not associated with gross rearrangements of the endogenous c-mos sequences in cellular DNA. The c-mos sequence does not contain any intervening sequences and is practically identical to the v-mos sequence. C-mos was not transcribed into RNA in any cells studied. These studies do not rule out the possibility that transient expression of c-mos sequences did occur during early transformation, or that other cellular oncogenic genes might play a role in cell transformation induced by chemical carcinogens or radiation. (43 Refs)

28. DNA METHYLATION AND GENE EXPRESSION: ENDOGENOUS RETROVIRAL GENOME BECOMES INFECTIOUS AFTER MOLECULAR CLONING.

Harbers K, Schnieke A, Stuhlmann H, Jahner D, Jaenisch R
Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie, Universitat Hamburg, Martinistrasse 52, 2000 Hamburg 20, W. Germany
Proc Natl Acad Sci USA; 78(12):7609-7613 1981

The Mov-3 substrain of mice carries Moloney murine leukemia virus as a Mendelian gene in its germ line. All mice segregating the Mov-3 locus activate virus and develop viremia and leukemia. The integrated provirus (ie, Mov-3 locus) was molecularly cloned from Mov-3 liver DNA as a 16.8 kilobase long EcoRI fragment. Comparison of the cloned and genomic Mov-3 specific EcoRI fragment by restriction enzyme analysis showed no differences in the size of the fragments, indicating that no major sequence rearrangements occurred during cloning. The genomic and cloned Mov-3 DNAs were compared for methylation and infectivity. Analysis with Hha I showed that the genomic proviral and the flanking mouse sequences were methylated at cytosine residues, in contrast to the cloned Mov-3 locus. The cloned Mov-3 locus, however, was highly infectious in a transfection assay (1 x 10(-5) plaque-forming unit per viral genome) in contrast to the genomic Mov-3 DNA (less than 10(-7) per viral genome). Our results suggest that genes containing 5-methylcytosine are not expressed after transfection into susceptible cells and that removal of the methyl groups by molecular cloning in prokaryotes leads to expression generating infectious proviral DNA. If gene expression of transfected DNA is controlled by mechanisms that are relevant for gene expression in the animal, this suggests that DNA methylation may play a causative role in eukaryotic gene regulation. (Author abstract) (35 Refs)

29. LONG TERMINAL REPEAT OF MURINE RETROVIRAL DNAS: SEQUENCE ANALYSIS, HOST-PROVIRAL JUNCTIONS, AND PREINTEGRATION SITE.

Van Beveren C, Rands E, Chattopadhyay SK, Lowy DR, Verma IM
(c/o Verma) Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
J Virol; 41(2):542-556 1982

The nucleotide sequence of the long terminal repeat (LTR) of three murine retroviral DNAs has been determined. The data indicate that the U5 region (sequences originating from the 5' end of the genome) of various LTRs is more conserved than the U3 region (sequences from the 3' end of the genome). The location and sequence of the control elements such as the 5' cap, 'TATA-like' sequences, 'CCAAT-box,' and presumptive polyadenylic acid addition signal AATAAA in the various LTRs are nearly identical. Some murine retroviral DNAs contain a duplication of sequences within the LTR ranging in size from 58 to 100 base pairs. A variant of molecularly cloned Mo-

loney murine sarcoma virus DNA in which one of the two LTRs integrated into the viral DNA was also analyzed. A four-base-pair duplication was generated at the site of integration of LTR in the viral DNA. The host-viral junction of two molecularly cloned AKR-murine leukemia virus DNAs (clones 623 and 614) was determined. In the case of AKR-623 DNA, a three- or four-base-pair direct repeat of cellular sequences flanking the viral DNA was observed. However, AKR-614 DNA contained a five-base-pair repeat of cellular sequences. The nucleotide sequence of the preintegration site of AKR-623 DNA revealed that the cellular sequences duplicated during integration are present only once. Finally, a striking homology between the sequences flanking the preintegration site and viral LTRs was observed. (Author abstract) (44 Refs)

30. THE U3 PORTION OF FELINE LEUKEMIA VIRUS DNA IDENTIFIES HORIZONTALLY ACQUIRED PROVIRUSES IN LEUKEMIC CATS.

Casey JW, Roach A, Mullins JI, Burck KB, Nicolson MO, Gardner MB, Davidson N
(c/o Davidson) Dept. Chemistry, California Inst. Technology, Pasadena, CA, 91125
Proc Natl Acad Sci USA; 78(12):7778-7782 1981

The presence and location of DNA sequences related to the U3 and U5 portions of the infectious exogenous feline leukemia virus (FeLV) long terminal repeat (LTR) in various cat DNAs have been determined by hybridization experiments. In uninfected cat DNAs, the U5 LTR segment from the Gardner-Arnstein strain B virus is present at approx 150 copies per cell. This level is approx 10-fold greater than that of endogenous internal FeLV sequences. The U5 sequences differ in copy number and, to some extent, in location from one animal to another. For any one animal, the sequence organization of the U5 segments is the same among different tissues, showing that the pattern is inherited through the germ line. Most importantly, the viral U3 LTR probe hybridizes only very weakly with uninfected cat DNAs. Both the U3 and the U5 regions of the LTR from the Gardner-Arnstein strain of virus cross-hybridize with DNA derived from four other infectious FeLVs representing A, B, and C subtypes. Thus, the U3 region may be used as a probe for studying the number and location of exogenously acquired FeLV proviruses in infected cat tissues. In some cases exogenously acquired proviruses are present in unique sites in the genome of virus-positive cat lymphosarcomas, indicating a monoclonal origin for the tumor. In other tumors, the proviral sequences are randomly distributed over many sites. Lymphosarcomas of virus-negative cats have no exogenous U3 sequences despite epidemiological evidence of an association of virus-negative leukemia with exposure to FeLV. (Author abstract) (17 Refs)

31. COMPARATIVE RESTRICTION ENDONUCLEASE MAPS OF PROVIRAL DNA OF THE PRIMATE TYPE C SIMIAN SARCOMA-ASSOCIATED VIRUS AND GIBBON APE LEUKEMIA VIRUS GROUP.

Trainor CD, Wong-Staal F, Reitz MS
(c/o Reitz) Div. Cancer Treatment, Lab. Tumor Cell Biology, NCI, Bethesda, MD, 20205
J Virol; 41(1):298-308 1982

Extrachromosomal DNA was purified from canine thymus cells acutely infected with different strains of infectious primate type C viruses of the woolly monkey (simian) sarcoma helper virus and gibbon ape leukemia virus group. All DNA preparations contained linear proviral molecules of 9.1 to 9.2 kilobases, at least some of which represent complete infectious proviral DNA. Cells infected with a replication-defective fibroblast-transforming sarcoma virus and its helper, a replication-competent nontransforming helper virus, also contained a 6.6-to 6.7-kilobase DNA. These proviral DNA molecules were digested with different restriction endonucleases, and the resultant fragments were oriented to the viral RNA by a combination of partial digestions, codigestion with more than one endonuclease, digestion of integrated proviral DNA, and hybridization with 3'-and 5'-specific viral probes. The 3'- and 5'-specific prob es each hybridized to fragments from both ends of proviral DNA, indicating that, in common with those of other retroviruses, these proviruses contain a large terminal redundancy at both ends, each of which consists of sequences derived from both the 3' and 5' regions of the viral RNA. The proviral sequences are organized 3',5'-unique-3',5'. Four restriction enzymes (KpnI, SmaI, PstI, and SstI) recognized sites within the large terminal redundancies, and these sites were conserved within all the isolates tested. This suggests that both the 3' and 5' ends of the genomic RNA of these viruses are extremely closely related. In contrast, the restriction sites within the unique portion of the provirus were not strongly conserved within this group of viruses, even though they were related along most of their genomes. Whereas the 5' 60 to 70% of the RNA of these viruses was more closely related by liquid hybridization experiments than was the 3' 30 to 40%, restriction sites within this region were not preferentially conserved, suggesting that small sequence differences or point mutations or both exist throughout the entire unique portion of the genome among these viruses. (Author abstract) (37 Refs)

32. ORGANIZATION AND EXPRESSION OF ENDOGENOUS VIRUS-LIKE (VL30) DNA SEQUENCES IN NONTRANSFORMED AND CHEMICALLY TRANSFORMED MOUSE EMBRYO CELLS IN CULTURE.

Courtney MG, Schmidt LJ, Getz MJ
(c/o Getz) Dept. Cell Biology, Mayo Clinic/Foundation, Rochester, MN, 55905
Cancer Res; 42(2):569-576 1982

A cloned mouse DNA fragment containing an endogenous 'virus-like' DNA (VL30 [illegible]) sequence was identified by virtue of its ability to hybridize to the virus-like RNA component of mixed-pseudotype AKR-murine leukemia virus virions, its lack of detectable sequence homology with cloned AKR-murine leukemia virus DNA, and its hybridization to a 5.6 kilobase pair (30S) cellular polyadenylic acid [poly(A)]-containing RNA species. Restriction enzyme mapping of the cloned mouse fragment revealed the presence of a 5-to 6-kilobase pair VL30 DNA segment flanked by non-VL30 segments of approx 7 and 0.3 kilobase pairs. Southern blot analysis of VL30 DNA sequence organization in the DNA of two nontransformed mouse cell lines (AKR-2B, C3H/10T1/2) and two chemically transformed derivatives (AKR-MCA, C3H/MCA-58) revealed 15 to 20 bands organized in an apparent strain-specific pattern. Within a given strain, however, no differences were detectable between the nontransformed cells and their chemically transformed counterparts. The expression of VL30 genes in the above cell lines was assayed by hybridization of 32P-labeled poly(A)-containing polysomal RNA to several internal restriction fragments derived from the cloned VL30 DNA sequence. The level of VL30 RNA was enhanced approx 10-fold in both chemically transformed cell lines as compared to the nontransfor med cell lines (under normal growth conditions). In addition, nontransformed AKR-2B cells maintained in the presence of purified epidermal growth factor exhibited similarly enhanced levels of VL30 RNA sequences in polysomal RNA.

Since these cells display several growth characteristics of transformed cells but, in an epidermal growth factor-dependent and completely reversible fashion, these data suggest that the expression of VL30 genes is not simply incidental to chemically transformed cells but may be related to alterations in growth control. (Author abstract) (34 Refs)

33. CELL ONCOGENES ARE LOCATED ON THE LARGE MICROCHROMOSOMES IN CHICKEN CELLS.

Tereba A, Lai MM
Div. Virology, St. Jude Children's Res. Hosp., Memphis, TN, 38101
Virology; 116(2):654-657 1982

The chromosomal locations of c-myc and c-myb, the cell homologs of the transforming genes present in myelocytomatosis virus 29, MC29, and avian myeloblastosis virus, AMV, respectively, were determined in White Leghorn chickens using in situ hybridization. Nucleic acid probes containing MC29 and AMV-RNAs were used to localize both c-myc and c-myb to a subset of large microchromosomes. The chromosomal location of c-myc was confirmed using nucleic acid probes containing RNA from MH-2, which also contains a myc-like sequence, and cloned DNA containing the chicken c-myc gene. In conjunction with our previous results localizing c-src and c-erb, we have demonstrated that several cell oncogene homologs appear to be localized on a subset of large microchromosomes. (Author abstract) (18 Refs)

34. TRANSFECTION OF FIBROBLASTS BY CLONED ABELSON MURINE LEUKEMIA VIRUS DNA AND RECOVERY OF TRANSMISSIBLE VIRUS BY RECOMBINATION WITH HELPER VIRUS.

Goff SP, Tabin CJ, Wang JY, Weinberg R, Baltimore D
(c/o Baltimore) Center Cancer Res., Massachusetts Inst. Technology, Cambridge, MA, 02139
J Virol; 41(1):271-285 1982

A cloned, permuted DNA copy of the Abelson murine leukemia virus (A-MuLV) genome was capable of eliciting the morphological transformation of NIH/3T3 fibroblasts when applied to cells in a calcium phosphate precipitate. The efficiency of the process was extremely low, yielding approx one transformant per microgram of DNA under conditions which give 10(4) transfectants per microgram of other DNAs (eg, Moloney sarcoma virus proviral DNA). The DNA was able to induce foci, even though the 3' end of the genome was not present. The transforming gene was thus localized to the 5' portion of the genome. The transformed cells all produced viral RNA and the virus-specific P90 protein. Transmissible virus could be rescued from these cells at very low frequencies by superinfection with helper virus; the rescued A-MuLV virus had variable 3' ends apparently derived by recombination with the helper. Dimerization of the permuted A-MuLV cloned genome to reconstruct a complete provirus did not improve transformation efficiency. Virus could be rescued from the transformants, however, at a high efficiency. Cotransfection of the permuted A-MuLV DNA with proviral M-MuLV DNA yielded a significant increase in the efficiency of transformation and cotransfection of dimeric A-MuLV and proviral M-MuLV resulted in a high-efficiency transformation yielding several thousand more transformants per microgram than A-MuLV DNA alone. We propose that helper virus efficiently rescues A-MuLV from transiently transfected cells which would not otherwise have grown into foci. We hypothesize that multiple copies of A-MuLV DNA introduced into cells by transfection are toxic to cells. In support of this hypothesis, we have shown that A-MuLV DNA sequences can inhibit the stable transformation of cells by other selectable DNAs. (Author abstract) (38 Refs)

ANTIVIRALS

35. A REVIEW OF APPROACHES TO VIRAL CHEMOTHERAPY.

Gordon M, Buchanan RL, Crenshaw RR, Kerridge KA, MacNintch JE, Siminoff P
Bristol Lab., Syracuse, NY, 13201
J Med; 12(5):289-384 1981

Progress in the field of viral chemotherapy is reviewed. The following topics were discussed: impact of viral diseases; viral morphology, physiology, replication, and pathogenicity; viral biochemistry; synthetic antiviral agents; recent developments in interferon research; immune stimulants, and future prospects. Role of viruses in oncogenesis is illustrated by the transmission of avian leukemia and Rous sarcoma. Other viruses involved in leukemia, sarcoma, and carcinoma were isolated from primates, cows, hamsters, horses, rats, and cats. DNA-containing oncogenic viruses include papovaviruses, adenoviruses, herpesviruses, and poxviruses; RNA-containing tumor-inducing viruses include the avian leukemia-sarcoma complex, the murine leukemia-sarcoma complex, and the primate oncornaviruses. Neoplastic cell transformation after virus infection can be characterized by by changes in cell appearance, increase in cellular metabolism, increase in multiplication rate, and loss of contact inhibition. (370 Refs)

36. MEASUREMENT OF INTERFERON EFFECTS ON MURINE LEUKEMIA VIRUS (MULV)-INFECTED CELLS.

Pitha PM
Biochemical Virology Lab., Johns Hopkins Oncology Center, Baltimore, MD, 21205
Methods Enzymol; 79:422-432 1981

Assays of infectious murine leukemia virus (both ecotropic and xenotropic), suitable for measuring interferon effects, are reviewed. Cell-free virus assay, infectious centers assay, in situ infectious assay, and syncytia induction in XC cells (rat tumor line) are described for ecotropic viruses. For xenotropic viruses, the assay for MSV (murine sarcoma virus), virion-associated reverse transcriptase, radioisotope labeling of virus particles, and virus purification, are discussed. Chromatographic purifica tion of viruses, isolation of virus RNA and its analysis by gel electrophoresis, and separation and identification of virion proteins are reviewed. Assays of viral RNA and protein in infected cells, including methods of preparation, nucleic acid hybridization, northern blotting technique, and competitive radioimmunoassay, are also reviewed. (32 Refs)

37. INTRACELLULAR PRODUCTION OF VIRUS PARTICLES AND VIRAL COMPONENTS IN NIH/3T3 CELLS CHRONICALLY INFECTED WITH MOLONEY MURINE LEUKEMIA VIRUS: EFFECT OF INTERFERON.

Aboud M, Kimchi R, Bakhanashvili M, Salzberg S
Microbiology and Immunology Unit, Faculty of Health Sciences, Ben-Gurion, Univ. Negev, Beer-Sheva, Israel
J Virol; 40(3):830-838 1981

The effect of interferon on the biochemical properties and the maturation process of intracellular viral particles isolated

from the cytoplasmic fraction of NIH/3T3 cells chronically infected with Moloney murine leukemia virus was investigated. By labeling these virions with either [35S]methionine or [3H]glucosamine, we demonstrated that they contain the same viral proteins and glycoproteins found in extracellular virions. Interferon treatment was found to reduce the rate of intracellular virus assembly. This effect was not a consequence of an interferon inhibition of viral RNA synthesis or its translation or a consequence of an interference with the posttranslational cleavage processing of viral precursor proteins, since all of these steps were not affected by interferon. However, the reduced rate of virus assembly could be attributed to the inhibition of viral protein glycosylation observed in interferon-treated cells. Nevertheless, despite this reduced rate, virus particles accumulated in interferon-treated cells. This accumulation was probably due to the strong inhibition of their final release from such cells. (Author abstract) (39 Refs)

38. RAPID SYNCYTIUM FORMATION INDUCED BY MOLONEY MURINE SARCOMA VIRUS IN 3T3/NIH CELLS AND ITS DELAY BY MOUSE INTERFERON.

Aboud M, Huleihel M
Unit Microbiology and Immunology, Faculty Health Sciences, Ben Gurion Univ. Negev, Beer Sheba, Israel
Arch Virol; 70(2):103-114 1981

Moloney murine sarcoma virus (MSV) clone 124 was found to induce rapid syncytium formation upon infection of 3T3/NIH at a high multiplicity of infection. This effect became apparent, by light microscopy, within 1 to 2 hr, whereas by scanning electron microscopy, clusters of 4 to 25 cells were seen in their initial steps of syncytium formation within 20 to 30 min after virus addition. It appeared that the cell fusion was initiated by connection between the cells through virus bound to their surface. After a few more hr several neighboring syncytia fused into giant cells containing over a hundred nuclei. Though MSV (124) stocks contained also some MLV it appeared that the syncytium inducing activity was related to the MSV part icles. MLV particles were not only incapable to induce syncytium formation, they even interfered with this activity of MSV. The MSV-induced cell fusion required the adsorption of intact virions, but was independent on protein synthesis. Mouse interferon remarkably reduced the rate of syncytium development. (Author abstract) (40 Refs)

39. LACK OF INTERFERON-INDUCED TRANSFER OF VIRAL RESISTANCE AGAINST MURINE LEUKEMIA VIRUS OR POLIOVIRUS IN COCULTIVATED CELLS.

Mozes LW
Papanicolaou Cancer Res. Inst., Miami, FL, 33101
Virology; 116(1):359-362 1982

It has recently been reported that there can be a transfer of the interferon (IFN)-induced viral resistance between cocultivated animal cells. However, in those systems the challenge virus was capable of replicating in both cell types. A more convincing conclusion could be obtained if the infecting virus could not replicate in the cell type which is homologous to the species of IFN that is added to the cultures. An inhibition of the yield of this virus would indicate that there was a transfer of the antiviral state from one cell species to another. In cocultivated mouse and human cells, a murine ecotropic Type-C virus can only multiply in the mouse cells whereas poliovirus will only replicate efficiently in the human cells. It is clearly evident from this study that only homologous but not heterologous fibroblast IFN is capable of inhibiting the replication of either of these viruses in various combinations of cocultivated cells. (Author abstract) (13 Refs)

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VIRAL PROTEINS

1. PROCESSING AND AMINO ACID SEQUENCE ANALYSIS OF THE MOUSE MAMMARY TUMOR VIRUS ENV GENE PRODUCT.

Arthur LO, Copeland TD, Oroszlan S, Schochetman G
Biological Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD, 21701
J Virol; 41(2):414-422 1982

The envelope proteins of mouse mammary tumor virus (MMTV) are synthesized from a subgenomic 24S mRNA as a 75,000-dalton glycosylated precursor polyprotein which is eventually processed to the mature glycoproteins gp52 and gp36. In vivo synthesis of this env precursor in the presence of the core glycosylation inhibitor tunicamycin yielded a precursor of approximately 61,000 daltons (P61env). However, a 67,000-dalton protein (P67env) was obtained from cell-free translation with the MMTV 24S mRNA as the template. To determine whether the portion of the protein cleaved from P67env to give P61env was removed from t he NH2-terminal end of P67env and as such would represent a leader sequence, the NH2-terminal amino acid sequence of the terminal peptide

HE 20.3173/2:

gp52 was determined. Glutamic acid, and not methionine, was found to be the amino-terminal residue of gp52, indicating that the cleaved portion was derived from the NH2-terminal end of P67env. The NH2-terminal amino acid sequences of gp52's from endogenous and exogenous C3H MMTVs were determined through 46 residues and found to be identical. However, amino acid composition and type-specific gp52 radioimmunoassays from MMTVs grown in heterologous cells indicated primary structure differences between gp52's of the two viruses. The nucleic acid sequence of cloned MMTV DNA fragments in conjunction with the NH2-terminal sequence of gp52 allowed localization of the env gene in the MMTV genome. Nucleotides coding for the NH2 terminus of gp52 begin approximately 0.8 kilobase to the 3' side of the single EcoRI cleavage site. Localization of the env gene at that point agrees with the proposed gene order -gag-pol-env- and also allows sufficient coding potential for the glycoprotein precursor without extending into the long terminal repeat. (Author abstract) (39 Refs)

2. BIOCHEMICAL CHARACTERIZATION OF PROTEIN KINASE ACTIVITIES ASSOCIATED WITH TRANSFORMING GENE PRODUCTS OF THE SNYDER-THEILEN AND GARDNER-ARNSTEIN STRAINS OF FELINE SARCOMA VIRUS.

Snyder HW
Lab. Viral Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
Virology; 117(1):165-172 1982

Translation products of the genomes of the Snyder-Theilen (ST) and Gardner-Arnstein (GA) feline sarcoma viruses (FeSV) have been shown to possess associated protein kinase activities. These activities are similar to those associated with a number of rapidly transforming retroviruses in that they appear to mediate transfer of radiolabel from [gamma-32P]ATP to selected tyrosine residues in substrates and to be independent of the effects of cAMP and cGMP in vitro. The FeSV activities, when detected in specific immune complexes bound to Staphylococcus aureus, result predominantly in autophosphorylation of FeSV polyproteins. Partially purified polyprotein also induces phosphorylation of an exogenous acceptor (casein) in a soluble protein kinase assay system. The FeSV enzymes display marked preference for Mn++ and Co++ over Mg++ while Ca++ fails to initiate activity in vitro. The optimal pH for the reactions is near 7.0. There is a strict requirement for ATP as phosphoryl donor. High concentrations of ADP and dADP are inhibitory to the reaction but low concentrations of dADP are stimulatory. A comparison of these properties with those of other retroviral-associated protein kinases suggests that these activities fall into three, and possibly four, classes. (Author abstract) (52 Refs)

3. BACTERIAL EXPRESSION OF AN ENZYMATICALLY ACTIVE PROTEIN ENCODED BY RSV SRC GENE.

McGrath JP, Levinson AD
Dept. Molecular Biology, Genentech, Incorporated, 460 Point San Bruno Blvd., South San Francisco, CA, 94080
Nature; 295(5848):423-425 1982

A bacterial plasmid was constructed which is capable of expressing in Escherichia coli a protein (p60) closely related in size and immunological properties to a 60,000-molecular wt (Mr) phosphoprotein (pp60src). The p60 protein appeared to be the translational product of the src gene under the control of the trp promoter. Total cellular proteins were analyzed in E coli transformed with recombinant vector pBR322, pHGH207-1, or pSRCex16. No overall change in the protein profile occurred in cells transformed with pBR322. In cells transformed with pHGH207-1, a prominent protein of 22,000 Mr appeared. Only in pSRCex16-transformed cells, was a 60,000 Mr p60 protein induced; p60 appeared selectively during late growth phase. When induced, p60 represented approx 5% of total bacterial protein. Further characterization of the protein was performed. Protein kinase (PK) activity was assayed in recombinant E coli strain harboring either pBR322 or the p60 expression plasmid (pSRCex16). PK activity was present only in bacteria expressing p60; PK activity, when expressed, was tyrosine-specific. The finding of a tyrosine-specific PK activity in a highly purified bacterially-derived preparation of p60 lended support to the conclusion that this enzymatic activity represents an intrinsic property of the molecule. (35 Refs)

4. EXPRESSION OF TRANSFORMING REGION OF MOLONEY MURINE SARCOMA VIRUS IN ESCHERICHIA COLI AS A FUSION PROTEIN WITH SMALL TUMOR ANTIGEN OF POLYOMA VIRUS.

Donoghue DJ, Hunter T
Tumor Virology Lab., The Salk Inst., P.O. Box 85800, San Diego, CA, 92138
Proc Natl Acad Sci USA; 79(3):800-804 1982

Bacterial expression of the transforming region of Moloney murine sarcoma virus, designated mos, was obtained as a fusion protein with a portion of the small tumor antigen of polyoma virus. This was accomplished by fusing the entire mos open reading frame, encoding a 41,000-dalton protein, with a plasmid that expresses a beta-galactosidase-polyoma fusion protein under lac operon control. The resulting plasmid directed synthesis of the predicted polyoma antigen-sarcoma virus fusion protein of 59,000 daltons. This protein was immunoprecipitated by an antipolyoma tumor antigen antiserum that recognized polyoma determinants at the NH2 terminus of the hybrid protein. This protein was also immunoprecipitated by an antiserum directed against a synthetic peptide containing the 12 COOH-terminal amino acids encoded by the mos open reading frame. This work confirms the existence of a long open reading frame in the mos gene and resolves a discrepancy between different nucleotide sequences for its COOH-terminal coding region. (Author abstract) (32 Refs)

VIRAL RNA

5. ELECTRON MICROSCOPIC CHARACTERIZATION OF AVIAN MYELOBLASTOSIS VIRUS RNA BY THE NON-PROTEIN TECHNIQUE OF SPREADING.

Jochmanova V, Korb J
Inst. Molecular Genetics, Czechoslovak Acad. Sciences, 166 37 Praha, Czechoslovakia
Folia Biol (Praha); 27(6):369-378,XXVIII-XXXI 1981

Avian myeloblastosis virus (AMV) RNA isolated by sodium dodecyl sulfate-phenol extraction from purified virions was denatured by 80% formamide at 53 C for 30 sec or by buffered 1.5% formaldehyde at 37 C for 2 min. Electron microscopic preparations were either (1) ramp spread with 2.5 x 10(-3)% benzyldimethylalkylammonium chloride (BAC) or 30 ug/ml cytochrome c or (2) coated by drop technique with 1 x 10(-3)% BAC or 0.01% anthrabis on Parlodion or carbon film. Under strongly denaturing conditions AMV RNA molecules

spread on monomolecular film of BAC by the ramp technique were significantly longer than those distributed over protein films; treatment of BAC films by glow discharge in air resulted in significantly longer, smooth molecules, and a similar effect was produced by changing the surface potential of the supporting carbon film with ethidium bromide. Results with the drop technique were similar, but reproducibility was poor. With the gentle denaturation of formaldehyde and spreading from 30% formamide, BAC spreading yielded measurable molecules with occasional 'kinky' structures, but cytochrome c gave molecules so aggregated as to be unmeasurable. The formaldehyde-formamide technique was applied to visualizing the complex formed by the specific viral protein p-12 and AMV-RNA. (19 Refs)

6. ISOLATION OF A TRANSFORMATION-DEFECTIVE DELETION MUTANT OF MOLONEY MURINE SARCOMA VIRUS.

Evans LH, Duesberg PH
(c/o Duesberg) Dept. Molecular Biology, Univ. California, Berkeley, CA, 94720
J Virol; 41(2):735-743 1982

A transformation-defective (td) deletion mutant of Moloney murine sarcoma virus (td Mo-MSV) and a transforming component termed Mo-MSV 3 were cloned from a stock of clone 3 Mo-MSV. To define the defect of the transforming function, the RNA of td Mo-MSV was compared with those of Mo-MSV 3 and of another transforming variant termed Mo-MSV 124 and with helper Moloney murine leukemia virus (Mo-MuLV). The interrelationships among the viral RNAs were studied by fingerprinting and mapping of RNase T1-resistant oligonucleotides (T1-oligonucleotides) and by identification of T1-oligonucleotides present in hybrids formed by a given viral RNA with cDNA's made from another virus. A comparison of td Mo-MSV sequences with either Mo-MSV 124 or Mo-MSV 3 indicated directly, in a fashion similar to the deletion analyses which defined the src gene of avian sarcoma viruses, that Mo-MuLV-unrelated sequences of Mo-MSV are necessary for transformation. A definition of transformation-specific sequences of Mo-MSV by d eletion analysis confirmed and extended previous analyses which have identified Mo-MuLV-unrelated sequences in Mo-MSV RNA and other studies which have described transformation of mouse 3T3 fibroblasts upon transfection with DNAs containing the Mo-MSV-specific sequence. (29 Refs)

7. GENOME STRUCTURE OF THE DEFECTIVE AVIAN SARCOMA VIRUS PRCIV.

Wong TC, Hirano A, Lai MM, Vogt PK
(c/o Vogt) Dept. Microbiology, Univ. Southern California Sch. Medicine, 2011 Zonal Ave., Los Angeles, CA, 90033
Virology; 117(1):156-164 1982

The genome of PRCIV, a defective avian sarcoma virus of class II, was studied by nucleic acid hybridization and oligonucleotide mapping. It has a length of about 6.1 kilobases (kb). A stretch of retrovirus replicative sequences encompassing part of the gag, all of the pol, and most of the env region, in all about 5 kb, is deleted. In its place a 2.9 kb substitution that is transformation-specific for PRCIV was found. Flanking the PRCIV-specific substitution are sequences shared with the helper virus, a stretch of 2.0 kb on the 5'-side, including part of the gag region, and a stretch of up to 1 kb on the 3'-side, including the C-region and possibly some env sequences. A PRCIV-specific cDNA probe representing the transformation-specific sequences was prepared. This probe hybridizes only with RNAs of other class II sarcoma viruses but fails to hybridize with either class I or class III sarcoma viruses. (Author abstract) (25 Refs)

8. CHARACTERIZATION OF GENOME STRUCTURE OF AMPHOTROPIC AND ECOTROPIC WILD MOUSE RETROVIRUSES.

Lai MM, Shimizu CS, Rasheed S, Pal BK, Gardner MB
Dept. Microbiology, Univ. Southern California Sch. Medicine, Los Angeles, CA, 90033
J Virol; 41(2):605-614 1982

We studied the RNA genomes of several wild mouse Type C retroviruses by using RNase T1-oligonucleotide fingerprinting. The amphotropic and ecotropic viruses of field strain 1504 produced very similar oligonucleotide fingerprints, but each also had several unique oligonucleotides. All of these unique oligonucleotides were located in the env gene region and were probably responsible for the host range differences between these viruses, as well as the lymphomagenic and paralytogenic properties of the viruses. We obtained similar results with the amphotropic and ecotropic viruses of another field strain (4070), which was isolated from a mouse from a different trapping area. The amphotropic viruses of several field strains (strains 1504, 4070, and 1313) were more closely related than the ecotropic viruses of different strains (strains 1504, 4070, and 4996). These findings suggested that the genetic sequences of the amphotropic viruses are more conserved than those of ecotropic viruses isolated from the same wild mice. (Author abstract) (26 Refs)

9. GENOMIC CHARACTERIZATION OF A HIGHLY ONCOGENIC ENV GENE RECOMBINANT BETWEEN AMPH OTROPIC RETROVIRUS OF WILD MOUSE AND ENDOGENOUS XENOTROPIC VIRUS OF NIH SWISS MOUSE.

Lai MM, Rasheed S, Shimizu CS, Gardner MB
Dept. Microbiology, Univ. Southern California Sch. Medicine, Los Angeles, CA, 90033
Virology; 117(1):262-266 1982

A highly oncogenic retrovirus (strain 10A/1) recovered from NIH Swiss mice inoculated with wild mouse amphotropic virus (strain 1504A) was studied by T1-oligonucleotide fingerprinting of the RNA genome. The virus was found to be an env gene recombinant between the parental 1504A and endogenous AT124 or related xenotropic virogenes of NIH Swiss mice. (Author abstract) (15 Refs)

10. RESTRICTED EXPRESSION OF INTEGRATED PRIMATE TYPE-C VIRUS (GIBBON APE LEUKEMIA VIRUS-SIMIAN SARCOMA VIRUS) PROVIRAL DNA IN NONPRODUCTIVELY INFECTED HUMAN B LYMPHOBLASTS.

Ceccherini Nelli L, Dalla-Favera R, Markham PD, Ruscetti FW, Wong-Staal F, Gallo RC, Reitz MS
(c/o Gallo) Lab. Tumor Cell Biology, NCI, NIH, Bethesda, MD, 20205
Virology; 117(1):195-206 1982

Human B-lymphoblast cell lines established from peripheral blood after infection with retroviruses of the simian sarcoma/simian sarcoma associated virus (SSV/SSAV)-gibbon ape leukemia virus (GALV) group generally do not produce detectable virus, even though viral RNA and protein are expressed. We show here by Southern blot and liquid molecular hybridization experiments that most of these nonproducer cultures

not producing virus contain apparently complete proviral DNA sequences which lack detectable rearrangements, indicating that restriction of virus replication is not due to obvious proviral lesions. The provirus is often present in only a fraction of the cell population, although the proviral copy number is variable and increases during extensive tissue culture or passage through nude mice. The proviral DNA is integrated into multiple heterogeneous sites in these cells, indicating a polyclonal population. A few of these cell lines have been cloned. Clonal lines have so far originated only from cells which contain proviral DNA and express viral RNA, although they still do not produce complete or infectious virus. Southern blots show that these cells are clonal in origin and contain a complete provirus with two LTRs arranged in the normal orientation. Two viral mRNA species are expressed which are identical in size to those from virus-producing cell lines. The smaller (presumably env) mRNA, like that from virus-producing lines is a spliced transcript which contains both 5'- and 3'-derived sequences. These data strongly suggest that these cells restrict viral replication at some stage later than mRNA synthesis, possibly at the level of protein synthesis or processing. (Author abstract) (15 Refs)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

11. ACTIVATION OF SV40 GENOME BY 72-BASE PAIR TANDEM REPEATS OF MOLONEY SARCOMA VIRU S.

Levinson B, Khoury G, Woude GV, Gruss P
Lab. Molecular Virology, NCI, NIH, Bethesda, MD, 20205
Nature; 295(5850):568-572 1982

The simian virus 40 (SV40) 72-base pair (bp) tandem-repeated sequences have a crucial role as an activator element in viral gene expression. We placed the SV40 72-bp repeat with a 72-bp repeat derived from the long terminal repeat (LTR) of cloned Moloney murine sarcoma virus (MSV) DNA. Although there is no detectable sequence homology to SV40, the MSV repeats can substitute functionally for the SV40 repeats and generate a viable virus in monkey kidney cells. (Author abstract) (45 Refs)

12. REVERSE TRANSCRIPTION OF AVIAN MYELOBLASTOSIS VIRUS 35S RNA. EARLY SYNTHESIS OF PLUS STRAND DNA OF DISCRETE SIZE IN RECONSTRUCTED REACTIONS.

Olsen JC, Watson KF
Dept. Chemistry, Univ. Montana, Missoula, MT, 59812
Nucleic Acids Res; 10(3):1009-1027 1982

The early DNA products of reverse transcription have been analyzed from reconstructed reactions containing avian myeloblastosis virus 35S RNA-tRNAtrp complex and highly purified reverse transcriptase. We describe conditions for the synthesis of genome-length complementary DNA and two discrete species of plus strand DNA (the same chemical polarity as the viral RNA genome) about 300 and 400 nucleotides in length. Plus DNA400 and plus DNA300 were detected by molecular hybridization with DNA probes complementary to sequences from both the 3'- and 5'-ends of the viral RNA. Both species appear to be copied from the 5'-end of minus strand DNA by their hybridization properties and their early synthesis when only the 5'-end of minus strand DNA is available as template. Restriction endonuclease mapping of plus DNA400 and plus DNA300 rules out a precursor-product relationship between the two. Rather the results suggest a unique initiation site for both species, with plus DNA400 containing internal sequences not present in plus DNA300. Plus DNA400 and plus DNA300 appear to be analogous to early plus DNA species detected in cells early after retrovirus infection. Thus, purified reverse transcriptase appears to be enzymatically sufficient for synthesis of genome-length complementary DNA and initiation and synthesis of early plus strand DNA as observed in infected cells. (Author abstract) (54 Refs)

13. DELETIONS OF SPECIFIC REGIONS OF THE SIMIAN SARCOMA-ASSOCIATED VIRUS GENOME ARE FOUND IN DEFECTIVE VIRUSES AND IN THE SIMIAN SARCOMA VIRUS.

Gelmann EP, Petri E, Cetta A, Wong-Staal F
Lab. Tumor Cell Biology, NCI, Bethesda, MD, 20205
J Virol; 41(2):593-604 1982

Extrachromosomal DNA was isolated from tissue culture cells that were acutely infected with simian sarcoma virus (SSV) and its associated helper (simian sarcoma-associated virus [SSAV]). Two sizes of closed circular viral genomic DNA intermediates were isolated, cleaved at the single EcoRI site, and ligated to the Charon 21A phage lambda vector. Cloned molecules of the larger size all represented the full-length (9.0-kilobase [kb]) SSAV molecule. A heterogeneous group of clones was derived from the smaller DNA circles. These included the SSV genome and SSAV deletion mutants. When two SSV clones were compared with the helper, they contained the following three characteristic deletions: (i) a 250-base pair deletion in the gag gene about 1.0 kb from the 5' end of the genome; (ii) a 1.85-kb deletion in the pol gene; and (iii) a 1.9-kb deletion at the 3' end, which included part of the env gene. This latter deletion was the site of the onc gene substitution. Six other clones of the smaller molecules represented the following variants of the SSAV genome: (i) two clones of the entire genome containing only one long terminal repeat unit; (ii) one clone with the 1.85-kb deletion of the pol gene observed in SSSV; and (iii) three clones having a deletion of the 3' end of the SSAV genome. In each of the latter clones, the 5' border of the deletion was indistinguishable from the 5' border of the onc substitution in SSV. The fidelity of genetic deletions observed suggested that certain regions of the SSAV genome were deleted at a high frequency. In certain cases, these deletions may have been accompanied by a substitution of cellular sequences to generate SSV. (Author abstract) (27 Refs)

14. ORGANIZATION OF TYPE C VIRAL DNA SEQUENCES ENDOGENOUS TO BABOONS: ANALYSIS WITH CLONED VIRAL DNA.

Battula N, Hager GL, Todaro GJ
Lab. Viral Carcinogenesis, NCI, Bethesda, MD, 20205
J Virol; 41(2):583-592 1982

Unintegrated linear and circular forms of baboon endogenous Type C virus M7 DNA were prepared from M7-infected cells by chromatography on hydroxyapatite columns, and the circular DNAs were purified in cesium chloride-ethidium bromide equilibrium density gradients. The circular DNAs were linearized by digestion with EcoRI, which had a unique site on the viral DNA. The linearized DNA was then inserted into lambdagtWES-lambdaB at the EcoRI site and cloned in an approved EK2 host. Molecularly cloned full-length M7 DNA was restricted with BamHI, and the resulting five subgenomic fragments were then subcloned individually in plasmid

pBR322. The organization and sites of integration of the approx 100 copies of M7 DNA sequences endogenous to baboons were investigated by digesting the DNA with restriction enzymes and identifying the virus-specific fragments by hybridization to labeled probes made by using the molecularly cloned full-length and subgenomic fragments of the viral DNA. We found that most of the endogenous sequences had sizes and organizations similar to those of the unintegrated viral DNA and therefore approx similar to the RNA of the infectious virus. A few of the multiple sequences had deletions in the 3' end (e nvelope region), and some of the sequences either lacked or contained modified BamHI restriction sites on the 5' end of the viral DNA. The endogenous viral DNA sequences were nontandem, uninterrupted, and colinear with the DNA of the infectious virus, and they were integrated at different sites in the baboon DNA, like the M7 proviral DNA sequences acquired upon infection. (Author abstract) (41 Refs)

15. RESTRICTION ENDONUCLEASE MAPPING OF UNINTEGRATED PROVIRAL DNA OF KIRSTEN MURINE SARCOMA VIRUS.

Norton JD, Carter AT, Avery RJ
(c/o Avery) Dept. Biological Sciences, Univ. Warwick, Coventry CV4 7AL, England
J Gen Virol; 58(part1):95-106 1982

Linear unintegrated Kirsten murine sarcoma virus DNA synthesized after acute infection of NIH-3T3 cells or by detergent-disrupted virions, was studied by restriction enzyme cleavage and agarose gel electrophoresis. Labeled DNA (cDNA) synthesized by reverse transcription of a virion RNA template was used to detect viral DNA sequences by filter hybridization. The sites of cleavage for 11 enzymes were located on the genome. Hybridization studies using a cDNA probe specific for the 3' end of the genome defined the orientation of the physical map with respect to virion RNA and identified terminally redundant sequences on the genome. Further evidence for terminal redundancy was obtained by restriction mapping of linear and circular viral DNA, where the duplication was shown to be 0.34 x 10(6) (500 base pairs) in length and in a tandem orientation. Circular viral DNA was found predominantly in the nucleus of acutely infected cells, and it existed in two major size classes. The larger size class represented an exact circularization of linear DNA (with no other sequence permutation) while in the smaller DNA species one of the terminally redundant sequences was absent. (Author abstract) (43 Refs)

16. CHARACTERIZATION OF PROVIRUSES CLONED FROM MINK CELL FOCUS-FORMING VIRUS-INFECTED CELLULAR DNA.

Khan AS, Repaske R, Garon CF, Chan HW, Rowe WP, Martin MA
Lab. Molecular Microbiology, Natl. Inst. Allergy and Infectious Diseases, Bethesda, MD, 20205
J Virol; 41(2):435-448 1982

Two proviruses were cloned from EcoRI-digested DNA extracted from mink cells chronically infected with AKR mink cell focus-forming (MCF) 247 murine leukemia virus (MuLV), using a lambda phage host vector system. One cloned MuLV DNA fragment (designated MCF 1) contained sequences extending 6.8 kilobases from an EcoRI restriction site in the 5' long terminal repeat (LTR) to an EcoRI site located in the envelope (env) region and was indistinguishable by restriction endonuclease mapping for 5.1 kilobases (except for the EcoRI site in the LTR) from the 5' end of AKR ecotropic proviral DNA. The DNA segment extending from 5.1 to 6.8 kilobases contained several restriction sites that were not present in the AKR ecotropic p rovirus. A 0.5-kilobase DNA segment located at the 3' end of MCF 1 DNA contained sequences which hybridized to a xenotropic env-specific DNA probe but not to labeled ecotropic env-specific DNA. This dual character of MCF 1 proviral DNA was also confirmed by analyzing heteroduplex molecules by electron microscopy. The second cloned proviral DNA (designated MCF 2) was a 6.9-kilobase EcoRI DNA fragment which contained LTR sequences at each end and a 2.0-kilobase deletion encompassing most of the env region. The MCF 2 proviral DNA proved to be a useful reagent for detecting LTRs electron microscopically due to the presence of nonoverlapping, terminally located LTR sequences which effected its circularization with DNAs containing homologous LTR sequences. Nucleotide sequence analysis demonstrated the presence of a 104-base-pair direct repeat in the LTR of MCF 2 DNA. In contrast only a single copy of the reiterated component of the direct repeat was present in MCF 1 DNA. (Author abstract) (44 Refs)

17. FUNCTION OF THE RETROVIRUS LONG TERMINAL REPEAT.

Temin HM
McArdle Lab., Univ. Wisconsin, Madison, WI, 53706
Cell; 28(1):3-5 1982

The long terminal repeat (LTR), a series of nucleotides derived from the 5' and 3' ends of viral RNA and located at the end of all infectious retrovirus DNA, is described. Information obtained from studies of the morphological transformation of NIH/3T3 cells after treatment with DNA containing LTR sequences and cellular homologs of viral transforming genes (oncogenes) is presented. Other data about LTR has been obtained from recombination of LTR from mouse mammary tumor virus with coding sequences. The presence of LTR has been used to explain differences in leukemogenicity of recombinant avian leukosis viruses. Evidence for the role of the ends of the LTR in processes related to integration is described. The LTR appears to contain most of the sequences involved in making retroviruses autonomous replicating elements. The relationship among viral, cellular, and LTR DNA is considered. (no Refs)

18. LONG TERMINAL REPEATS OF ENDOGENOUS MOUSE MAMMARY TUMOR VIRUS CONTAIN A LONG OPEN READING FRAME WHICH EXTENDS INTO ADJACENT SEQUENCES.

Kennedy N, Knedlitschek G, Groner B, Hynes NE, Herrlich P, Michalides R, Van Ooyen AJ
Kernforschungszentrum Karlsruhe, Institut fur Genetik und Toxikologie von Spaltstoffen, Postfach 3640, D7500 Karlsruhe 1, W. Germany
Nature; 295(5850):622-624 1982

The integrated DNA copies (proviruses) of RNA tumor virus genomes are flanked by long terminal repeat (LTR) sequences, which are generated by the duplication of segments present at both ends of the viral RNA. LTRs vary in length from 330 to 1,328 base pairs (bp). The primary viral transcript starts in the left LTR (L-LTR) and terminates in the right LTR (R-LTR). We have now sequenced both LTRs from an inherited (endogenous) mouse mammary tumor virus (MMTV) of the GR strain. This provirus, which is one of five present in the haploid genome of GR mice, does not produce virus and is not identical with the exogenous MMTV of GR mice. The provirus (GR40) could nevertheless be transcribed and its transcription hormonally regulated after cloning and transfection

into mouse L cells. The LTRs are identical and exhibit the following features: a terminal inverted six-bp repeat, a Goldberg-Hogness (TATAAA) sequence, sequences which have been implicated in polyadenylation and the termination of transcription, and an open reading frame. The open reading frame of the R-LTR starts at the env/LTR junction and encodes a 36,700-mol wt (Mr) protein. The open reading frame of the L-LTR extends into the adjacent mouse sequence. A host sequence of six bp at the site of integration into the mouse chromosome is directly repeated. (Author abstract) (31 Refs)

19. AN AVIAN TUMOR VIRUS PROMOTER DIRECTS EXPRESSION OF PLASMID GENES IN ESCHERICHIA COLI.

Mitsialis SA, Young JF, Palese P, Guntaka RV
(c/o Guntaka) Dept. Microbiology, Coll. Physicians and Surgeons, Columbia Univ., New York, NY, 10032
Gene; 16(1-3):217-225 1981

A sequence from the long terminal repeat (LTR) of DNA from avian tumor virus (ATV) was isolated. The LTR sequence contained a promoter that was active in Escherichia coli. When bacterial promoters for genes coding for resistance to tetracycline and neomycin were deleted from different plasmids and replaced by fragments from ATV DNA, recombinant plasmids expressed the drug-resistant phenotype dependent on an intact nucleotide sequence from nucleotide +19 to nucleotide-23. The drug-resistance expressed was equal to or greater than that expressed by bacterial promoters. (18 Refs)

20. MOLECULAR PROPERTIES OF A GAG- POL- ENV+ MURINE LEUKEMIA VIRUS FROM CULTURED AKR LYMPHOMA CELLS.

Rein A, Lowy DR, Gerwin BI, Ruscetti SK, Bassin RH
Biological Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD, 21701
J Virol; 41(2):626-634 1982

We have described the isolation of a replication-defective murine leukemia virus from a culture of AKR lymphoma cells. To facilitate the characterization of this murine leukemia virus, we transmitted it to mink cells and analyzed its genome by restriction mapping of the mink cellular DNA. This genome resembled the Akv genome quite closely, but it had an additional KpnI cleavage site at 1.3 kilobase pairs from the 5' end of the provirus and a small (approx 50-base-pair) deletion between 1.8 and 3.0 kilobase pairs from the 5' end. When we tested these mink cells by immune precipitation or by competition radioimmunoassay, we found that they synthesized gPr82env, but contained no detectable gag or pol proteins. It seems likely that the KpnI cleavage site at 1.3 kilobase pairs reflects an abnormal sequence at or near the beginning of the gag gene, which prevents gag or pol translation by introducing a frameshift or termination codon into this region. (Author abstract) (33 Refs)

21. MOLONEY MURINE LEUKEMIA VIRUS-INDUCED TUMORS: RECOMBINANT PROVIRUSES IN ACTIVE CHROMATIN REGIONS.

van der Putten H, Quint W, Verma IM, Berns A
Departement de Biologie Animale, Laboratoire de Differenciation Cellulaire, 154, Route de Malagnou, CH-1224, Chene-Bougeries, Geneve, Switzerland
Nucleic Acids Res; 10(2):577-592 1982

The DNase I sensitivity of chromosomal DNA regions carrying integrated proviral genomes of Moloney (M-MuLV) and AKR Murine Leukemia Virus (AKR-MuLV), and the cellular homologue of the mos-gene (c-mos) of Moloney Sarcoma Virus (MSV) were studied in tumor tissues of leukemic mice. The genetically transmitted sequences of M-MuLV, AKR-MuLV, and the c-mos gene are all in DNase I resistant chromatin conformations in M-MuLV-induced tumors. Each M-MuLV-induced tumor contained at least one somatically acquired integrated recombinant MuLV genome that displayed two main characteristic features of active chromatin; (a) a configuration hypersensitive to DNase I, and (b) extensive hypomethylation. DNase I hypersensitive sites were mapped at the junction of cellular sequences and the 5'-viral large terminal repeat (LTR). Expression of a recombinant MuLV seems therefore to be a necessary feature to maintain the transformed state. (Author abstract) (28 Refs)

22. ISOLATION AND CHARACTERIZATION OF CHICKEN DNA HOMOLOGOUS TO THE TWO PUTATIVE ONCOGENES OF AVIAN ERYTHROBLASTOSIS VIRUS.

Vennstrom B, Bishop JM
Dept. Microbiology, Univ. Uppsala, Biomedical Center, Uppsala, Sweden
Cell; 28(1):135-143 1982

The genome of avian erythroblastosis virus contains two independently expressed genetic loci (v-erbA and v-erbB) whose activities are probably responsible for oncogenesis by the virus. Both loci are closely related to nucleotide sequences found in the DNA and RNA of chickens and other vertebrates. We have isolated and characterized chicken DNA homologous to v-erbA and v-erbB. The two viral genes are represented by separate domains within chicken DNA (c-erbA and c-erbB), which are separated by a minimum of 12 kilobases (kb) of DNA and may not be linked at all. The nucleotide sequences shared by the viral and cellular erb loci are colinear, but the cellular loci are interrupted by multiple intervening sequences of various lengths. Polyribosomes prepared from normal chicken embryos contain two polyadenylated RNAs transcribed from c-erbA and two transcribed from c-erbB. The evident coding regions of these RNAs represent an unusually small fraction of the lengths of the RNAs, as if the 3' untranslated domains of the RNAs might be exceptionally large (3-11 kb). These findings indicate that the c-erb loci are normal vertebrate genes rather than genes of cryptic endogenous retroviruses, and that they may have a role in the metabolism of normal cells. It appears that the viral erb genes, like most other retrovirus oncogenes, have been copied from cellular genes. In the viral genome, the two genes are devoid of introns, but they remain independently expressed loci, and they remain colinear with the coding domains of their cellular progenitors. (Author abstract) (30 Refs)

23. STRUCTURAL RELATIONSHIP BETWEEN A NORMAL CHICKEN DNA LOCUS AND THE TRANSFORMING GENE OF THE AVIAN ACUTE LEUKEMIA VIRUS MC29.

Robins T, Bister K, Garon C, Papas T, Duesberg P
Dept. Molecular Biology, Univ. California, Berkeley, CA, 94720
J Virol; 41(2):635-642 1982

We screened a recombinant chicken DNA/lambda phage library for sequences homologous to the transformation-specific sequences of the avian acute leukemia virus MC29 by hybridization with molecularly cloned MC29 proviral DNA. Three cellular DNA clones were found and compared with each other and with the viral genome by physical mapping with re-

striction endonucleases and by heteroduplex analysis. These experiments indicated that the three cellular clones overlap and represent a single cellular locus. The RNA genome of MC29 and normal cell DNA share a homologous region of 1.6 kilobases which is interrupted in the cellular DNA by 1.0 kilobase of sequences not present in the viral genome. Hybridization of the cloned cellular DNA to viral RNA and analysis of the protected viral RNA by fingerprinting techniques indicated that there is extensive sequence homology between the helper virus-unrelated mcv sequences of the viral RNA and the cellular DNA, with only minor base differences. The cellular mcv locus, however, lacks all helper virus-related sequences of MC29, including those of the partial viral gag gene which, together with mcv, encodes the probable transforming protein of MC29. We conclude that although the mcv locus of the normal cell does not represent a complete structural homolog to the onc gene of MC29, it is probably the precursor to the onc-specific sequence in the virus. (Author abstract) (27 Refs)

24. PRIMATE ORIGIN OF THE CELL-DERIVED SEQUENCES OF SIMIAN SARCOMA VIRUS.

Robbins KC, Hill RL, Aaronson SA
Lab. Cellular and Molecular Biology, Natl. Cancer Inst., Bethesda, MD, 20205
J Virol; 41(2):721-725 1982

We sought to identify the species of origin of the cell-derived (sis) sequences of simian sarcoma virus. A molecular clone comprised of sis DNA detected related nucleotide sequences at low copy numbers in normal cellular DNAs of species as diverse as humans and quail. The extent of hybridization and degree of base-pair matching with sis DNA were greatest with New World primate DNAs. The thermal den aturation curve midpoints of hybrids formed between sis and woolly monkey DNAs were indistinguishable from homologous sis DNA hybrids, establishing the woolly monkey (Lagothrix spp) as the source of sis sequences. In comparative studies, sis was shown to be more conserved among mammalian species than unique-sequence woolly monkey cellular DNA. There was no detectable homology between cis and the cell-derived sequences of other fibroblast-transforming retroviruses. These findings indicate that sis is likely to be a unique onc gene among transforming retroviruses. (Author abstract) (35 Refs)

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AUGUST, 1982

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VIRAL PROTEINS

1. PRIMARY STRUCTURE ANALYSIS OF THE MAJOR INTERNAL PROTEIN P24 OF HUMAN TYPE C T-CELL LEUKEMIA VIRUS.

Oroszlan S, Sarngadharan MG, Copeland TD, Kalyanaraman VS, Gilden RV, Gallo RC
Biological Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD, 21701
Proc Natl Acad Sci USA; 79(4,part1):1291-1294 1982

A human type C retrovirus [human T-cell leukemia (lymphoma) virus; HTLV], recently isolated from young adult patients with cutaneous T-cell lymphoma or leukemia, was not detectably related to other known animal retroviruses in molecular hybridization studies, by comparison of reverse transcriptase and the major core protein p24. The p24 core protein was purified to homogeneity. The amino acid composition, the COOH-terminal amino acid, and the NH2-terminal amino acid sequence of the first 25 residues of this major internal structural protein were determined. These results were then compared to the know n structure of the internal core protein of other retroviruses. The compositional data reveal that HTLV p24 is chemically distinct from p30-p24 of other animal retroviruses,

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in agreement with the earlier immunological analyses. However, HTLV p24 shares the common NH2-terminal proline and COOH-terminal leucine of all mammalian type C viral p30s. In addition, like bovine leukemia virus (BLV), HTLV lacks the common prolylleucylarginine tripeptide and the larger conserved region found near the NH2 terminus of the other mammalian type C viral p30s. Alignment of the amino acid sequence of HTLV p24 with previously determined sequences of other retrovirus proteins, including BLV p24, reveals statistically significant sequence homology only to BLV. The results reported here demonstrate that HTLV p24 is related to but chemically distinct from the major core protein of other retroviruses. Similarly, previous results showed that there was no immunological crossreactivity of the p24 protein and reverse transcriptase of HTLV with other retroviruses, including BLV, and no nucleic acid sequence homology. However, the present results, combined with the common size of the p24 and reverse transcriptase, suggest that HTLV may be closer to BLV than any other known retrovirus. (Author abstract) (23 Refs)

2. COMPLETE AMINO ACID SEQUENCE OF THE GROUP-SPECIFIC ANTIGEN GENE-ENCODED PHOSPHORYLATED PROTEINS OF MOUSE LEUKEMIA VIRUSES.

Versteegen RJ, Copeland TD, Oroszlan S
Biological Carcinogenesis Program, Frederick Cancer Res. Facility, Frederick, MD, 21701
J Biol Chem; 257(6):3007-3013 1982

The complete amino acid sequence of the group-specific antigen gene-encoded RNA binding phosphoprotein p12 has been determined for both Rauscher and Moloney leukemia viruses. Large fragments generated by acid, cyanogen bromide and hydroxylamine cleavage, and Staphylococcus aureus V8 protease digestion were subjected to automated sequencing. Both Rauscher and Moloney p12 are composed of 84 amino acids arranged in alternating variable and conserved regions. The homology between the conserved internal and COOH-terminal regions is greater than 95%, but the NH2-terminal and internal variable regions show 59 and 51% homology, respectively. The role of such regions in the type-specific biological activities of these molecules is discussed. (Author abstract) (42 Refs)

3. ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF PARTIALLY TRANSFORMATION-DEFECTIVE MUTANTS OF AVIAN MYELOCYTOMATOSIS VIRUS STRAIN MC29: LOCALIZATION OF THE MUTATION TO THE MYC DOMAIN OF THE 110,000-DALTON GAG-MYC POLYPROTEIN.

Ramsay GM, Hayman MJ
Imperial Cancer Res. Fund, London WC2A 3PX, England
J Virol; 41(3):745-753 1982

Recently, we isolated three mutants of MC29 virus which, although able to transform fibroblasts with the same efficiency as wild-type MC29, were 100-fold less efficient at transforming macrophages. In this study we found that MC29-transformed quail producer cell line Q10 was able to generate these partially transformation-defective mutants at a high frequency. Using tryptic peptide mapping, we determined that the smaller gag-myc polyproteins encoded by the transformation-defective viruses had lost myc-specific tryptic peptides. This suggested that the mutations which resulted in the transformation-defective viruses being inefficient at transforming macrophages were located in the v-myc sequence and thus directly implicated v-myc and the gag-myc polyprotein in transformation by MC29. (Author abstract) (35 Refs)

4. DNA-PROCESSING ACTIVITIES ASSOCIATED WITH THE PURIFIED ALPHA, BETA2, AND ALPHABETA5AB MOLECULAR FORMS OF AVIAN SARCOMA VIRUS RNA-DEPENDENT DNA POLYMERASE.

Hizi A, Gazit A, Guthmann D, Yaniv A
Dept. Cell Biology and Histology, Sackler Sch. Medicine, Tel-Aviv Univ., Tel-Aviv, Israel
J Virol; 41(3):974-981 1982

The RNA-dependent DNA polymerase purified from B77 avian sarcoma virus exhibited two distinct DNA-processing activity. The alpha and beta2 isoenzymes possessed an endodeoxyribonuclease activity capable of nicking simian virus 40 superhelical DNA, whereas the alphabeta isoenzyme performed as an untwisting topoisomerase. Both activities associated with the three molecular forms of the retroviral DNA polymerase were dependent on the presence of either $Mn++$ or $Mg++$ ions. From analysis of the denatured DNA products, it is apparent that the alpha and beta2 isoenzymes introduced two nicks, one per each strand in the superhelical simian virus 40 DNA molecules, whereas the alphabeta polymerase converted these supercoiled molecules to the relaxed covalently closed circular form. The notion that the DNA-processing activities are located on the DNA polymerase molecules was supported by the following: (i) the three isoenzymes were of a high purity; (ii) the activities cosedimented in glycerol gradients with the DNA polymerase activities of the alpha, beta2, and alphabeta molecular forms; and (iii) immunoglobulin directed against the purified polymerase immunoprecipitated the DNA-processing activities. Chemical treatments of the DNA polymerase molecules (with pyridoxalphosphate, iodoacetamide, and sulfhydryl reagents), which inhibited the polymerase activity, also suppressed the endonucleolytic and topisomerase activities, suggesting that cysteine and amino groups play an important role in the active sites of the DNA-processing activities as well. (Author abstract) (22 Refs)

5. REVERSE TRANSCRIPTASE ASSOCIATED WITH AVIAN SARCOMA-LEUKOSIS VIRUSES. I. COMPARISON OF INTRA-VIRION CONTENT OF MULTIPLE ENZYME FORMS.

Ueno A, Ishihama A, Toyoshima K
(c/o Ishihama) Inst. Virus Res., Kyoto Univ., Sakyo-ku, Kyoto, Kyoto 606, Japan
J Biochem (Tokyo); 91(1):311-322 1982

The RNA-dependent DNA polymerase (the reverse transcriptase) was solubilized from three related strains of avian sarcoma virus (ASV B77, ASV tsLA334, and ASV QV2) as well as avian myeloblastosis virus (AMV) and a chicken endogenous virus (RAV-0), by a combination of non-ionic detergent treatment and CsCl step-gradient centrifugation, and was subsequently separated into individual enzyme forms by poly(C)-agarose column chromatography. The newly developed two-step method allowed us to purify the three molecular forms (alpha, alphabeta-, and beta-form) of highly active enzyme rapidly and quantitatively from all the five virus strains examined. The molar ratio of the three enzyme forms differed among the virus strains: for the three sarcoma viruses, the major species was the alphabeta-form enzyme, the putative holoenzyme, and the alpha- and beta-form enzymes were less

than a few percent and 15-25%, respectively, while the alpha-form enzyme content was higher for the two leukosis viruses than for the three sarcoma viruses. Both the total DNA polymerase activity and the content of the two enzyme subunits in purified virions of the three sarcoma virus was in the following order: ASV tsLA334 greater than ASV B77 greater than ASV QV2, which paralleled the virus yield at a permissive temperature in roller bottle cultures of chick embryo fibroblasts. No alteration was found in the thermolability of DNA polymerases between tsLA334, which carries ts mutations affecting both virus growth and cell-transformation, and other viruses. (Author abstract) (30 Refs)

6. REVERSE TRANSCRIPTASE ASSOCIATED WITH AVIAN SARCOMA-LEUKOSIS VIRUSES. II. COMPARISON OF SUBUNIT STRUCTURE AND CATALYTIC PROPERTIES.

Ueno A, Ishihama A
(c/o Ishihama) Inst. Virus Res., Kyoto Univ., Sakyo-ku, Kyoto, Kyoto 606, Japan
J Biochem (Tokyo); 91(1):323-330 1982

The three enzyme forms (alpha, alphabeta-, and beta-form) of the RNA-dependent DNA polymerase (the reverse transcriptase) from three strains of avian sarcoma virus (ASV B77, ASV tsLA334, and ASV QV2) and one exogenous (avian myeloblastosis virus (AMV)) and one endogenous avian leukosis virus (Rous-associated virus type-0 (RAV-0)) were compared with each other in subunit structure and catalytic properties. Despite the gross similarity in the subunit mol wt (Mr(alpha) = 65,000 and Mr(beta) = 92,000), minor differences were found in the molecular size of the subunit as determined by SDS-gel electrophoresis, the order being ASV tsLA334 less than or equal to ASV QV2 less than ASV B77 less than or equal to RAV-O = AMV. The structural differences were supported by analysis of peptide fragments after treatment with S aureus V8 protease. Although the general catalytic properties of the purified enzymes from the five virus strains were similar, the selectivity of template-primer differed in the RAV-O enzymes. The template-primer selectivity of the reverse transcriptases from all five virus strains tested was also found to be greatly influenced by the reaction temperature for DNA synthesis, resulting in a temperature-dependent increase of poly(dG) synthesis over [(A)n].[(dT)12-18]-dependent poly(dT) synthesis. The RAV-O enzymes required a lower temperature for DNA synthesis, particularly for [(C)n].[(dG)12-18]-dependent poly(dG) synthesis. (Author abstract) (28 Refs)

7. REVERSE TRANSCRIPTASE ASSOCIATED WITH AVIAN SARCOMA-LEUKOSIS VIRUSES. III. INFLUENCE OF PRIMER CHAIN LENGTH ON THE INITIATION OF DNA SYNTHESIS.

Fukui T, Ishihama A, Ohtsuka E, Ikehara M, Fukuda R
(c/o Ishihama) Inst. Virus Res., Kyoto Univ., Sakyo-ku, Kyoto, Kyoto 606, Japan
J Biochem (Tokyo); 91(1):331-339 1982

The influence of oligodeoxynucleotide chain length on the priming activity of the reverse transcription was investigated in poly(A)-dependent poly(dT), poly(C)-dependent poly(dG) and influenza virus RNA-dependent cDNA synthesis by the alpha-beta-form enzyme of the RNA-dependent DNA polymerase from avian sarcoma-leukosis viruses. The minimum chain length required for the priming activity to be exposed varied depending on the nucleotide sequence of the primer and thus of the primer-binding sequence of the template RNA, ie, a sudden and max increase of the priming activity was observed at a primer chain length of between 2 and 4 for poly(dG) and between 6 and 8 for poly(dT) synthesis. The observed difference in the minimum chain length seems to parallel the difference in physical stability between poly(C)-oligo(dG) and poly(A)-oligo(dT) hybrids, although the primer chain lengths are shorter than those required for the respective hybrids paired at the reaction temperature. In influenza virus RNA-dependent cDNA synthesis, the priming activity of oligonucleotides complementary to the 3' terminal sequence of vRNA segments was enhanced at a chain length of between 10 and 12, maintaining a plateau thereafter. This unexpected requirement for long primers in influenza cDNA synthesis might reflect the unique conformation of vRNA, which forms a panhandle-like structure through intramolecular pairing between 3' and 5' termini. Phosphorylation of the 5' terminus inactivated oligo(dT) to a level of a few percent of the 5'-hydroxyl oligo(dT) in priming poly(A)-dependent poly(dT) synthesis, implying that the 5' phosphate interferes with the priming activity of oligonucleotides by a yet unidentified mechanism. However, the observed interference decreased concomitantly with increase of primer chain length. (Author abstract) (33 Refs)

8. IMMUNOCHEMISTRY OF THE MOLONEY MULV GP71 MOLECULE - I. DISTRIBUTION OF ANTIGENIC DETERMINANTS ON THE PRODUCTS OF DIGESTION BY STAPHYLOCOCCUS AUREUS PROTEASE.

Alonso JL, Rubio N
(c/o Rubio) Instituto de Immunologia y Biologia Microbiana, C.S.I.C., Velazquez, 144 Madrid 6, Spain
Mol Immunol; 19(1):65-73 1982

The antigenic characterization of Moloney murine leukemia virus gp71 was carried out by cleavage with Staphylococcus aureus protease. The whole molecule and two fragments of 43,000 and 32,000 daltons were immunoprecipitated with monospecific anti-type, group and interspecies antiserum. The three native molecules were recognized by anti-type antibodies, but only the gp71 and the 32,000-dalton molecule seemed to retain group-specific antigenic determinants. No interspecies determinants were detected on the two products of digestion. A similar pattern of precipitation was found with SDS-denatured antigens. The results suggest that sequential structures contribute to the type-specific antigenic determinants, because they were not destroyed by SDS unfolding or by the change in the protein conformation that follows enzymatic cleavage. Interspecies determinants seem to be mainly conformational, since they disappeared from the whole molecule and the two peptides after the same treatment. Group-specific determinants appear to be intermediate; they disappeared after the conformational change on the native 43K but not on the 32K peptide and the monospecific anti-group antibody showed an intermediate ability to precipitate the SDS-unfolded antigens. (Author abstract) (36 Refs)

9. RADIOIMMUNOLOCALIZATION OF MURINE MAMMARY TUMOR VIRUS PROTEINS IN GELS.

Bausch JN, Prasad KS, Spiegelman S
(c/o Spiegelman) Inst. Cancer Res., Coll. Physicians and Surgeons, Columbia Univ., New York, NY, 10032
Anal Biochem; 119(2):293-298 1982

A procedure for the immunological detection of antigens on sodium dodecyl sulfate-polyacrylamide gels using iodinated antibodies combined with an immunoconcentration step to enrich for the desired antigen(s) is described. This selective enrichment is achieved by separation of the antigen-antibody

complexes with formalin-treated cells of Staphylococcus aureus and is followed by radioimmunolocalization on gels. This method is particularly useful for the characterization of antigens present at low concentrations in complex biological fluids. Its usefulness is exemplified here by the successful identification of mouse mammary tumor virus antigens in extracts of murine mammary tumors and in plasmas of tumor-bearing animals. In particular, the procedure permits the ready determination that the gp52 protein detected in the plasmas of tumor-bearing animals is in fact present as a 52,000-dalton species and not as a higher mol wt precursor. (Author abstract) (17 Refs)

10. ONCOGENES.

Bishop JM
Univ. California, San Francisco, CA
Sci Am; 246(3):80-84,87-92,154 1982

Recent developments in cancer research concerning inducement of cancerous growth are reviewed. Some tumor viruses (TV) are oncogenic only in animals that are not hosts in nature, others are oncogenic only in their natural host. Transformation of cultured cells by TV makes possible the examination of the interaction of a TV with a host cell under controlled conditions, thus avoiding the difficulties associated with experiments in animals. Some viruses have a single gene which can induce tumors, or in some cases, a few such 'oncogenes'. Retroviruses (RV) are the only TV with an RNA genome. The src oncogene of Rous sarcoma virus is discussed. The protein encoded by src is a 60,000 mol wt phosphoprotein. Phosphorylation of tyrosine is a common characteristic of oncogene-encoded enzymes and also has a role in regulating the growth of normal cells. The mutational hypothesis proposes that viral oncogenes differ from their cellular progenitors as a result of mutations introduced when the cellular genes were copied into the RV genome. The dosage hypothesis suggests that RV oncogenes overburden cells with too much essentially normal proteins carrying out normal functions. Excessive activity might tip the balance of regulation toward incessant growth. In tumors induced by the chicken lymphoma viruses, the viral DNA is almost always inserted into cellular DNA in the immediate vicinity of a single cellular oncogene, and the expression of the cellular oncogene is greatly amplified. Gene-length bits of DNA isolated from some tumors can transmit the property of cancerous growth when they are introduced into previously normal cells in culture. (4 Refs)

11. NUCLEAR LOCALIZATION AND DNA BINDING OF THE TRANSFORMING GENE PRODUCT OF AVIAN MYELOCYTOMATOSIS VIRUS.

Donner P, Greiser-Wilke I, Moelling K
Max-Planck-Institut fur Molekulare Genetik, Berlin-Dahlem, Ihnestrasse 63-73, 1 Berlin 33, W. Germany
Nature; 296(5854):262-266 1982

A previous study has shown that the activated cellular gene in certain lymphomas was homologous to the oncogene of an acute avian leukemia virus, MC29. In MC29, the oncogene is fused to the viral structural gene, gag. The mol wt of the fused gene is 110,000 (110 K) and is designated as p110gag-myc. For further analysis, the 110 K protein was purified from the 35S-methionine-labeled MC29-transformed quail embryo fibroblast cell line MC29-Q8-NP (which does not produce virus particles, but expresses the transforming protein gag-myc) by an immune affinity column containing protein A-Sepharose to which monoclonal anti-p19 IgG was covalently coupled in conditions which link the constant region of the IgG molecule to the solid phase. Immunofluorescence studies and cell fractionation of MC29-transformed fibroblasts indicate that the 110 K protein is predominantly located in the nucleus. The purified protein was found to bind to double-stranded DNA. These properties may be related to the role of the protein in transformatio n. (22 Refs)

12. ANALYSIS OF THE SEQUENCE OF AMINO ACIDS SURROUNDING SITES OF TYROSINE PHOSPHORYLATION.

Patschinsky T, Hunter T, Esch FS, Cooper JA, Sefton BM
Tumor Virology Lab., Salk Inst., P. O. Box 85800, San Diego, CA, 92138
Proc Natl Acad Sci USA; 79(4,part1):973-977 1982

We have identified the single phosphorylated tyrosine in p60src, the transforming protein of Rous sarcoma virus, as part of the sequence NH2-Arg-Leu-Ile-Glu-Asp-Asn-Glu-Tyr(P)-Thr-Ala-Arg COOH. Therefore, this is a sequence that is recognized efficiently by a tyrosine protein kinase in vivo. Phosphorylation of tyrosine in cellular proteins appears to play a role in malignant transformation by four classes of genetically distinct RNA tumor viruses. Phosphorylated tyrosines in several other proteins resemble the tyrosine in p60src in that they are located 7 residues to the COOH-terminal side of a basic amino acid and either 4 residues to the COOH-terminal side of, or in close proximity to, a glutamic acid residue. Therefore, it is possible that these features play a role in the selection of sites of phosphorylation by some tyrosine protein kinases. However, several clear exceptions to this rule exist. (Author abstract) (38 Refs)

13. PHOSPHORYLATION AND METABOLISM OF THE TRANSFORMING PROTEIN OF ROUS SARCOMA VIRUS.

Sefton BM, Patschinsky T, Berdot C, Hunter T, Elliott T
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
J Virol; 41(3):813-820 1982

The transforming protein of Rous sarcoma virus, p60src, was found to contain 0.5 to 0.9 mol of total phosphate per mol of polypeptide. The protein is known to be phosphorylated at two sites, a serine in the amino-terminal domain and a tyrosine in the carboxy-terminal domain. Because our indirect analysis suggests that the serine is phosphorylated to approx twice the extent of the tyrosine, we estimate that p60src contains approx 0.3 to 0.6 mol of phosphoserine and 0.2 to 0.3 mol of phosphotyrosine per mol of polypeptide. p60src was found to represent approx 0.02% of the total incorporated radioactivity in Rous sarcoma virus-transformed chick cells labeled with [35S]methionine for 48 hr. This corresponds to approx 500,000 molecules of p60src per cell. Pulse-chase experiments revealed that the half-life of p60src ranged from 2 to 7 hr, depending on the strain of virus examined. The p60src of the Schmidt-Ruppin strain was significantly more stable than that of the Prague strain. (Author abstract) (25 Refs)

14. CELLULAR SUBSTRATE FOR THE SRC GENE PRODUCT.

Kobayashi N
Dept. Microbiological Medicinal Chemistry, Tokyo Univ Sch. Pharmacology, Tokyo, Japan
Tanpakushitsu Kakusan Koso; 26(10):1537-1553 1981

Current ideas concerning the phosphoprotein pp36K (mol wt 36,000), which is found in Rous sarcoma virus-infected cells, were presented. Its correspondence to viral carcinogenesis was discussed, and the following aspects were included bird sarco-

ma virus carcinogenic gene products, determination of src gene, src gene products, phosphorylation of p60src and its protein kinase activation, phosphorylation of thyroxin, and protein kinase activity carried by carcinogenic gene products other than src, intracellular marker proteins of src gene products, determination of pp36K, p36K (a non-phosphorylated precursor of pp36K), pp36K existing in cells other than chicken cells, in vitro phosphorylation of p36K, cellular elution using radio immune precipitation assay, unique damage in p36K phosphorylation reaction due to tumor-bearing rat serum, phosphorylated amino acids of pp36K, intracellular mechanism of pp36K, and intracellular marker proteins of pp60src other than p36K. (118 Refs) (In JPN)

VIRAL RNA

15. PARTICIPATION OF SUBGENOMIC RETROVIRAL MRNAS IN RECOMBINATION.

Wang LH, Stacey DW
Rockefeller Univ., New York, NY, 10021
J Virol; 41(3):919-930 1982

Envelope glycoprotein (env) mRNA from avian retroviruses was injected into cells transformed by env-deleted Bryan Rous sarcoma virus [RSV(-)]. The genetic deficiency of RSV(-) was complemented, and infectious transforming virus was released for many days after these injections. The long-term activity of the injected env mRNA is believed to be due to reverse transcription of the injected RNA after its incorporation into virus particles. The resulting subgenomic provirus, presumed to be integrated into host DNA, is able to direct the continuous synthesis of additional env mRNA. In some of these cultures, replication-competent viruses appeared many days after injection. The analysis by RNase T1 oligonucleotide fingerprinting showed that the RNA of these virus genomes contained oligonucleotides characteristic of both RSV(-) and the env mRNA injected. In all viruses analyzed the 5' two-thirds and a 3' terminus of the genome were derived from RSV(-) and the env gene from the injected mRNA. Our results thus strongly indicate that these viruses were generated via recombination between RSV(-) and env mRNA. The demonstration of involvement of an MRNA sequence in recombination may be of importance in the divergence of retroviruses and in the mechanism of interaction between retroviruses and host nucleotide sequences. (Author abstract) (32 Refs)

16. STUDIES ON THE FUNCTION OF THE NON-PRIMER TRNAS ASSOCIATED WITH THE 70S RNA OF AVIAN MYELOBLASTOSIS VIRUS.

Waters LC, Burke JR, Stulberg MP
Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
Biochim Biophys Acta; 696(2):201-207 1982

Significant amounts of three tRNAs are associated with the 70S RNA of avian myeloblastosis virus (AMV). The temperatures at which they are half dissociated from the 70S RNA in 50 mM NaCl and their respective quantities relative to 35S RNA are: tRNAArg, 51 C, 1.6; tRNALys, 57 C, 0.7 and t RNATrp, 76 C, 1.0. Possible functions for the non-primer tRNAs (tRNAArg and tRNALys) were evaluated by determining the effect of their thermal dissociation on: (a) conversion of 70S to 35S RNA, (b) capacity of 70S and/or 35S RNA to be translated in vitro, and (c) capacity of 70S and/or 35S RNA to be reverse transcribed in vitro. Conversion of 70S to 35S RNA occurred with a tm of 56 C and is consistent with the hypothesis that tRNALys might be involved in joining two 35S RNA subunits to form the 70S RNA complex. There was no indication that the association of either tRNAArg or tRNALys influenced the rate or quality of translation of 70S or 35S RNA. A decrease in the rate at which 70S RNA is transcribed occurs in parallel with the dissociation of tRNAArg and tRNALys. (Author abstract) (29 Refs)

17. AT LEAST TWO REGIONS OF THE VIRAL GENOME DETERMINE THE ONCOGENIC POTENTIAL OF AVIAN LEUKOSIS VIRUSES.

Robinson HL, Blais BM, Tsichlis PN, Coffin JM
Worcester Foundation Experimental Biology, Shrewsbury, MA, 01545
Proc Natl Acad Sci USA; 79(4,part1):1225-1229 1982

Recombinants of oncogenic and nononcogenic avian leukosis viruses were tested for their oncogenic potential in chickens. The results indicate that at least two regions of the viral genome determine the oncogenic potential of these viruses. The first region contains sequences that control viral mRNA synthesis. These sequences determine the potential of a virus to induce a low incidence of lymphomas, carcinomas, chondrosarcomas, fibrosarcomas, and osteopetrosis. The second region lies outside the sequences that control viral mRNA synthesis. These sequences determine the ability of a virus to induce a high incidence of lymphomas or osteopetrosis. (Author abstract) (41 Refs)

18. ALTERATIONS IN THE GENOMES OF AVIAN SARCOMA VIRUSES.

Mason WS, Linial M, Hsu TW, Eisenman RN, Townsend J, Mark GE, Seal G, Aldrich C, Taylor JM
Inst. Cancer Res., Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA, 19111
Virology; 117(2):456-474 1982

We describe 12 nonconditional mutants of avian sarcoma virus. Eleven were replication defective and 1 appeared to retain wild-type functions. Comparison of restriction endonuclease digests of viral DNA revealed that 8 of 11 replication defectives contained large deletions of viral information. A functionally wild-type variant contained an insertion of sequences between the env and src genes. The deletions appeared to be randomly distributed throughout the replicative genes: gag, pol, and env. In most, but not all, cases, the phenotype of the deletion mutants could be simply correlated with the location and extent of the deletions. In a few cases, the defects in expression appeared to be more extensive than would be expected from a single point or deletion mutation, suggesting that these mutants contained multiple mut ations or a single mutation in a region affecting several viral functions. (Author abstract) (52 Refs)

19. DELETIONS WITHIN THE TRANSFORMATION-SPECIFIC RNA SEQUENCES OF ACUTE LEUKEMIA VIRUS MC29 GIVE RISE TO PARTIALLY TRANSFORMATION-DEFECTIVE MUTANTS.

Bister K, Ramsay GM, Hayman MJ
Max-Planck-Institut fur Molekulare Genetik, D-1000 Berlin 33, W. Germany
J Virol; 41(3):754-766 1982

The viral RNAs of three nonconditional mutants of avian myelocytomatosis virus MC29 were analyzed. These mutants, which were originally isolated from the quail producer line

Q10 and were designated 10A, 10C, and 10H, have lost most of the ability to transform hematopoietic cells in vitro and to induce tumors in vivo, but they still transform cultured fibroblasts with the same efficiency as wild-type (wt) MC29. Electrophoretic analyses showed that the mutant genomic RNAs were smaller than the 5.7-kilobase genome of wt MC29; the genomes of mutants 10A, 10C, and 10H were about 5.5, 5.3, and 5.1 kilobases long, respectively. Analyses of the transformation-specific sequences of these mutant RNAs by a combination of T1 oligonucleotide fingerprinting and hybridization with cDNA from the transformation-specific sequences myc of wt MC29 or competition hybridization including wt MC29 RNA revealed that deletions of myc-specific sequences had occurred. The deletions in all three mutants overlapped, since they all had lost one particular myc-specific oligonucleotide. In agreement with the size of the genomic RNAs, mutants 10C and 10H had lost two additional myc oligonucleotides, and mutant 10A contained a modified myc oligonucleotide. The locations of the deletions were deduced from comparisons with previously established oligonucleotide maps of several members of the MC29 subgroup of acute leukemia viruses and by hybridization of wt and mutant RNAs to molecularly cloned subgenomic fragments of wt MC29 proviral DNA, representing the 5' and 3' domains of the myc sequence. We found that the deleted sequences represented overlapping internal segments of the myc sequence and that the borders of myc with the partial complements of the virion genes gag and env appeared to be conserved in mutant and wt MC29 RNAs. The correlation between the altered transforming potential for hematopoietic cells and the partial deletion of myc in the mutant RNAs provided direct genetic evidence for the involvement of myc in oncogenesis. However, the unaffected efficiency of these mutants in fibroblast transformation suggested that the deleted sequences are not essential for the fibroblast-transforming potential of the onc gene of MC29. (Author abstract) (31 Refs)

20. GENETIC STRUCTURE AND TRANSFORMING SEQUENCE OF AVIAN SARCOMA VIRUS UR2.

Wang LH, Hanafusa H, Notter MF, Balduzzi PC
Rockefeller Univ., New York, NY, 10021
J Virol; 41(3):833-841 1982

We have recently shown that a newly isolated avian sarcoma virus, UR2, is defective in replication and contains no sequences homologous to the src gene of Rous sarcoma virus. In this study, we analyzed the genetic structure and transforming sequence of UR2 by oligonucleotide fingerprinting. The sizes of the genomic RNAs of UR2 and its associated helper virus, UR2AV, were determined to be 24S and 35S, respectively, by sucrose gradient sedimentation. The mol wt of the 24S UR2 genomic RNA was estimated to be 1.1 x 10(6), corresponding to 3,300 nucleotides, by gel electrophoresis under the native and denatured conditions. RNase T1 oligonucleotide mapping indicated that UR2 RNA contains seven unique oligonucleotides in the middle of the genome and shares eight 5'- and six 3'-terminal oligonucleotides with UR2AV RNA. From these data, we estimated that UR2 RNA contains a unique sequence of about 1.2 kilobases in the middle of the genome, and contains 1.4 and 0.7 kilobases of sequences shared with UR2AV RNA at the 5' and 3' ends, respectively. Partial sequence analysis of the UR2-specific oligonucleotides by RNase A digestion revealed that there are no homologous counterparts to these oligonucleotides in the RNAs of other avian sarcoma and acute leukemia viruses studied to date. UR2-transformed non-virus-producing cells contain a single 24S viral RNA which is most likely the message coding for the transforming protein of UR2. On the basis of the uniqueness of the transforming sequence, we concluded that UR2 is a new member of the defective avian sarcoma viruses. (Author abstract) (37 Refs)

21. EXPRESSION OF THE PRC II AVIAN SARCOMA VIRUS GENOME.

Adkins B, Hunter T, Beemon K
Institut fur Virusforschung, Im Neuenheimer Feld 280, 6900 Heidelberg, W. Germany
J Virol; 41(3):767-780 1982

We found that the genomic RNA of the replication-defective avian sarcoma virus PRC II was 4.0 kilobases long. A Northern blot analysis of the viral RNAs present in PRC II-transformed cells showed that the PRC II genome was expressed as a single 4.0 kilobase mRNA species. In vitro translation of poly-adenylic acid-containing 70S virion RNA yielded two highly related proteins of 110,000 and 105,000 daltons (P110 and P105), which were synthesized from messenger activity that sedimented as expected for the 4.0 kilobase PRC II genome (at 25 to 27S). P110 and P105 were identified as in vitro translation products of the PRC II genome by immunoprecipitation and tryptic peptide mapping and were the only PRC II-specific polypeptides detected by in vitro synthesis. In addition, we found that immune complexes prepared from PRC II 70S virion RNA in vitro translation products contained a tyrosine-specific protein kinase activity. A comparison of the in vitro- and in vivo-synthesized proteins revealed that PRC II-transformed cells also contain ed 110,000- and 105,000-dalton proteins, which were indistinguishable from in vitro-synthesized P110 and P105 by electrophoretic mobility and tryptic peptide analysis. Both P110 and P105 were present in producer cells and in seven individual nonproducer clones. A pulse-chase analysis showed that P105 was the primary translation product of the PRC II genome and that P110 was derived from P105 by post-translational modification. Under conditions of long-term labeling with [35S]methionine, P110 and P105 were present in a molar ratio of approximately 1:1. These results indicated that the transformation-specific product of the PRC II genome, previously referred to as a single component (P105), actually consists of two polypeptides related by post-translational modification. (Author abstract) (36 Refs)

22. TRANSCRIPTS OF CELLULAR ANALOG OF VIRUS SARCOMA SRC GENE IN MOUSE LIVER.

Mett VL, Chumakov IM, Kiselev LL
Inst. Molecular Biology, Moscow, USSR
Dokl Akad Nauk SSSR; 261(6):1471-1472 1981

Analysis of the expression of protosarc gene in the liver of mice uninfected with Moloney sarcoma virus (MSV) showed synthesis of poly(A)-RNA homologous to RNA of MSV. Poly(A)-RNA showing hybridization with src gene of MSV were also detected in the liver of mice treated with the hepatocarcinogen o-aminoazotoluene. These findings can indicate transcription of the endogenous sarcoma provirus during chemical carcinogenesis. (7 Refs) (In RUS)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

23. SITE-SPECIFIC MUTAGENESIS BY ERROR-DIRECTED DNA SYNTHESIS.

Zakour RA, Loeb LA
(c/o Loeb) Joseph Gottstein Memorial Cancer Res. Lab., Dept. Pathology,, SM-30 Univ. Washington, Seattle, WA, 98195
Nature; 295(5851):708-710 1982

The use of misincorporation during DNA synthesis to create site-specific mutagenesis was attempted using single-stranded bacteriophage phiX174 circular DNA containing an amber mutation. The fragment HpaI2 (phiX174 positions 31-1,294) was obtained from phiX174 RF DNA by endonuclease cleavage. The 3'OH terminus of the fragment was elongated using DNA polymerase from Escherichia coli with deoxyATP (dATP) and deoxycytosine triphosphate (dCTP), which were complementary to the first three template positions. A second sunthesis phase utilized the error-prone DNA polymerase from avian myeloblastosis virus (AMV). Deoxynucleosides were used in three different combinations. After allowing for misincorporation, the subsequent correct nucleotide was inserted. The newly copied DNA was transfected into E coli, and the frequency of am18 mutations was determined. When dATP, which was complimentary to position 23 in the am18 region, was present during replication with AMV DNA polymerase, the reversion frequency was 4x that of uncopied template DNA. When dATP was not present, the reversion frequency was 100x times that of uncopied templat e DNA. Further experiments confirmed that reversions were caused by single-base substitutions. Thymine-guanine mispairing was more likely than thymine-thymine or thymine-cytosine mispairing. Results indicate that misincorporation can be used to create site-specific mutagenesis. (23 Refs)

24. DNA SEQUENCE RELATIONSHIP OF THE BABOON ENDOGENOUS VIRUS GENOME TO THE GENOMES OF OTHER TYPE C AND TYPE D RETROVIRUSES.

Cohen M, Rice N, Stephens R, O'Connell C
Biological Carcinogenesis Program, Frederick Cancer Res. Facility, Frederick, MD, 21701
J Virol; 41(3):801-812 1982

Baboon endogenous virus (BaEV) is a Type C retrovirus present in multiple proviral copies in the DNA of baboons. Although interspecies antigenic determinants present on reverse transcriptase and gag proteins are shared among all mammalian Type C viruses, no nucleic acid homology between BaEV and other Type C viruses (except RD-114) has been found in conventional liquid hybridization experiments. In this study, we used restriction fragments of cloned BaEV DNA immobilized on nitrocellulose to test for relatedness with [32P]cDNA's of various Type C and Type D viruses. We detected the following distant relationships previously found only through immunological and protein sequencing techniques: (i) eight Type C viral cDNA's and two Type D virus cDNA's were able to hybridize with cloned BaEV DNA; (ii) the eight Type C probes hybridized to restriction fragments spanning most of the BaEV genome, but only RD-114 hybridized to fragments within the 1.9 kilobases at the 3' end of the genome; (iii) the two Type D probes hybridized primarily to fragments within the 1.9 kilobases at the 3' terminus and weakly or not at all elsewhere; and (iv) [32P]cDNA's of several other oncornaviruses exhibited no homology with BaEV DNA. DNA sequence analysis has allowed us to orient the BaEV restriction map with the genetic map at both ends of the genome. Homologies between retroviral cDNA's and BaEV clone restriction fragments could thus be related to specific BaEV genes. These results are discussed relative to previously reported antigenic relatedness of retroviral proteins. The data suggest that BaEV represents an important link in oncornavirus evolution. (44 Refs)

25. COLOBUS TYPE C VIRUS: MOLECULAR CLONING OF UNINTEGRATED VIRAL DNA AND CHARACTERIZATION OF THE ENDOGENOUS VIRAL GENOMES OF COLOBUS.

Birkenmeier EH, Bonner TI, Reynolds K, Searfoss GH, Todaro GJ
Jackson Lab., Bar Harbor, ME, 04609
J Virol; 41(3):842-854 1982

The unintegrated viral DNA intermediates of colobus type C virus (CPC-1) were isolated from infected human cells that were permissive for viral growth. There were two major species of DNA, linear molecules with two copies of the long terminal repeat and relaxed circles containing only a single long terminal repeat. In addition, there was a minor species (approx 10%) composed of relaxed circles with two copies of the long terminal repeat. A restriction endonuclease map of the unintegrated DNA was constructed. The three EcoRI fragments of circular CPC-1 DNA were cloned in the EcoRI site of lambdagtWES-lambdaB and then subcloned in the EcoRI site of pBR322. Using these subgenomic fragments as probes, we have characterized the endogenous viral sequences found in colobus cellular DNA. They are not organized in tandem arrays, as is the case in some other gene families. The majority of sequences detected in cellular DNA have the same map as the CPC-1 unintegrated DNA at 17 of 18 restriction endonuclease sites. There are, however, other sequences that are present in multiple copies and do not correspond to the CPC-1 map. They do not contain CPC-1 sequences either in an altered form or fused to common nonviral sequences. Instead, they appear to be derived from a distinct family of sequences that is substantially diverged from the CPC-1 family. This second family of sequences, CPC-2, is also different from the sequences related to baboon endogenous type C virus that form a third family of virus-related sequences in the colobus genome. (Author abstract) (27 Refs)

26. HUMAN NUCLEOTIDE SEQUENCES RELATED TO THE TRANSFORMING GENE OF A MURINE SARCOMA VIRUS: STUDIES WITH CLONED VIRAL AND CELLULAR DNAS.

Chumakov IM, Zabarovsky ER, Prassolov VS, Mett VL, Kisselev LL
Inst. Molecular Biology, USSR Acad. Sciences, ul. Vavilova 32, Moscow V-334, USSR
Gene; 17(1):19-26 1982

Fragments of the human genome related to onc-gene of Moloney sarcoma virus (MSV), mos-specific, and Moloney leukemia virus (MLV)-specific nucleotide sequences were cloned. An attempt was made to visualize the expression of oncogenes by the Northern blotting technique, using a mos-specific probe, and preliminary data are presented. MSV clone 124 was isolated from tissue culture supernatant obtained from chronically infected TB cells. A total of 3 x 10(5) recombinant phages containing human DNA insert were subjected to screening. [32P]Complimentary (c)DNA-MSV was used as a probe and 30 labeled plaques were obtained. All of them were tested with the mos-specific probe, but with one exception, no mos-specific DNA was detected. After the whole screening procedure, 19 recombinant phages with inserts hybridizable with the mos-specific probe were selected. Clones isolated with various probes

could be subdivided into those that contain MLV-related sequences and lack mos-related sequences and those that contain both types of sequences. The hybridization pattern of polyadenylic+RNA from normal mouse liver with [32P]cDNA-MSV is presented. Several fractions were revealed which hybridize also with cDNA-MLV. These RNA appeared to be transcripts of endogenous proretroviruses homologous to MLV which probably are present in mouse liver. Whe n a mos-specific probe was used, no hybridization was detected with RNA isolated independently from several groups of mice. (32 Refs)

27. MOLECULAR CLONING OF DNA FRAGMENTS HOMOLOGOUS TO THE ONCOGEN OF MOUSE SARCOMA VIRUS FROM THE COLLECTION OF GENES OF THE TRANSFORMED CELLS.

Zabarovskii ER, Prasolov VS, Chumakov IM, Kiselev LL
Inst. Molecular Biology, Moscow, USSR
Dokl Akad Nauk SSSR; 261(6):1456-1459 1981

Molecular cloning of murine genes transformed with Moloney sarcoma virus (MSV) showed that sarc-gene is adjacent to the virus-specific nucleotide sequences. Analysis of the collection of molecular clones showed the absence of the provirus DNA identical to MSV genome. The isolated DNA fragments homologous to MSV oncogen were identified as the endogenous defective sarcoma proviruses. (12 Refs) (In RUS)

28. MOLECULAR CLONING OF MURINE ENDOGENOUS VIRAL SEQUENCES AND EXPRESSION OF A NEWLY CONSTRUCTED RECOMBINANT MURINE LEUKEMIA VIRUS DNA IN TRANSFECTED MINK CELLS.

van der Hoorn FA, Onnekink C, Van Der Putten H, Zijlstra M, Bloemers HP
Dept. Biochemistry, Univ. Nijmegen, Geert Grooteplein Noord 21, 6525 EZ Nijmegen, The Netherlands
Proc Natl Acad Sci USA; 79(5):1398-1402 1982

In the process of molecularly cloning unintegrated proviral DNA from NIH-3T3 mouse cells infected with Rauscher murine leukemia virus, we observed the occurrence of clones with inserts smaller than the expected Rauscher murine leukemia virus fragments. The insert of one of these clones, lambda.Xe-1, was characterized in more detail. It had a size of 3.5 kilobases. The restriction map was similar but not identical to that of the envelope regions of Moloney and Rauscher murine leukemia viruses. After ligation to previously cloned Moloney murine leukemia viral sequences and transfection of the ligated DNA into mink lung cells a nondefective xenotropic murine leukemia virus, XH-19, was isolated. Restriction mapping of proviral DNA isolated from mink lung cells chronically infected with XH-19 showed the presence of Moloney murine leukemia virus-derived sequences coupled to xenotropic viral sequences. (Author abstract) (24 Refs)

29. LONG-TERM PRESERVATION OF TRANSFECTING ACTIVITY OF AVIAN SARCOMA PROVIRAL DNA.

Ogura H, Fujiwara T
Dept. Virology, Cancer Inst., Okayama Univ. Medical Sch., Okayama 700, Japan
Acta Med Okayama; 35(5):377-379 1981

The integrated proviral DNA of avian sarcoma virus (ASV) in host cell chromosomes has been isolated and stored in saline sodium citrate (SSC) solution or in 70% ethanol at 4 C in a refrigerator over 4 yr. This DNA was assayed by transfection of chick embryo cells (CEC). The biological activity of cellular transformation by the stored DNA was compared with that of a fresh isolate of the proviral DNA. The efficiency of the transfection by each DNA was almost the same. (Author abstract) (7 Refs)

30. RESTRICTION ENDONUCLEASE MAPPING OF THE PROVIRAL DNA OF THE EXOGENOUS RIII MURINE MAMMARY TUMOR VIRUS.

Etkind PR, Szabo P, Sarkar NH
Lab. Molecular Virology, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
J Virol; 41(3):855-867 1982

Cellular DNA containing integrated murine mammary tumor virus (MuMTV) was isolated from Fel/C6 feline kidney cells and CCL64 mink lung cells infected with milkborne RIII MuMTV. By using restriction enzyme HpaI, intact RIII MuMTV provirus (length, 8.7 kilobases [kb]) was excised from the cellular DNA. Subsequent restriction endonuclease analysis of this HpaI fragment with KpnI, HindIII, EcoRI, BamHI, BglII, PstI, SstI, SalI, and XhoI enabled us to construct a map of the RIII virus genome. A comparison of this map with the maps of the GR and C3H MuMTV's revealed that there are greater sequence differences between the RIII virus and the GR and C3H MuMTV proviruses than there are between the GR and C3H proviruses. The following are features of the restriction map unique to the RIII provirus: the presence of three BamHI and two EcoRI cleavage sites, a HpaI cleavage site in the terminal 3'-5' repeat unit of the provirus, and the absence of an XhoI cleavage site. Another distinguishing feature of the RIII provirus is that the sizes of some of the restriction fragments produced by cleavage of the RIII provirus with PstI are different from the sizes of the fragments obtained by PstI cleavage of the GR and C3H proviruses. Like the GR proviral DNA, the RIII proviral DNA has three SstI (SacI) cleavage sites, whereas the C3H provirus has only two SstI sites. HpaI digestion of MuMTV-infected mink lung cell DNA revealed only one class of provirus (an 8.7-kb fragment); however, we observed minor classes of RIII proviral DNA in addition to the major class of provirus DNA in infected cat kidney cells. PstI digestion of the HpaI 8.7-kb fragments from both feline and mink cells generated a 3.7-kb DNA fragment identical in size to a PstI-generated fragment that has been found in GR and C3H milkborne virus-infected cells. Although a fragment similar in size to the milkborne 3.7-kb PstI fragment has been found an endogenous component fragment in the RIII mouse strain. Therefore, the 3.7-kb fragment may be useful as a marker for the milkborne RIII MuMTV provirus in RIII mice. (Author abstract) (50 Refs)

31. METHYLATION AND AMPLIFICATION OF MOUSE MAMMARY TUMOR VIRUS DNA IN NORMAL, PREMALIGNANT, AND MALIGNANT CELLS OF GR/A MICE.

Fanning TG, Vassos AB, Cardiff RD
Dept. Pathology, Sch. Medicine, Univ. California, Davis, CA, 95616
J Virol; 41(3):1007-1013 1982

The methylation and amplification of mouse mammary tumor virus (MuMTV) proviral DNA was investigated in normal, premalignant, and malignant tissues of GR/A mice. The proviral methylation pattern was examined with the restriction enzyme HhaI, which fails to cleave methylated DNA. MuMTV proviral DNA from liver, kidney, and heart was highly methylated. Proviral DNA was somewhat undermethylated in mammary gland cells from virgin and lactating mice

and extensively undermethylated in cells from premalignant outgrowths, pregnancy-dependent tumors, and pregnancy-independent tumors. The restriction enzyme SacI was used to detect additional proviruses in the same cells. No additional proviral copies of MuMTV were detected in liver, kidney, or heart cells or in mammary gland cells from virgin mice. Some mammary gland cells from lactating mice appeared to contain additional copies of the endogenous, highly oncogenic GR-MTV-2 provirus. Premalignant outgrowth, pregnancy-dependent tumor, and pregnancy-independent tumor cells contained an average of two to three additional copies per cell of the GR-MTV-2 provirus. Thus, neoplasia in GR/A mice was directly associated with quantized increases in MuMTV proviral DNA undermethylation and GR-MTV-2 proviral DNA amplification. Restriction enzyme analysis suggested that premalignant outgrowths and pregnancy-dependent tumors both consisted largely of heterogenous cell populations, although some evidence of clonal dominance was detected. (Author abstract) (31 Refs)

32. ORGANIZATION OF PROVIRAL DNA AND PROTEIN COMPOSITION OF HORMONALLY STIMULATED C57BL/10 STRAIN-ASSOCIATED MOUSE MAMMARY TUMOR VIRUS.

Svec J, Matoska J, Keszeghova V, Hlavayova E
Cancer Res. Inst., Slovak Acad. Sciences, 812 32 Bratislava, Czechoslovakia
Neoplasma; 28(6):647-659 1981

Two continuous lines, BL-MaTU/A1 and BL-MaTU/S6, were established from C57Bl/10 mammary adenocarcinomas induced by DMBA-prolactin-estradiol treatment. Under in vivo stimulation with dexamethasone, insulin, prolactin, and prostaglandin A1, the cells produce detectable amounts of B-type particles with biochemical properties similar to the GR-MuMTV. Analysis of restriction endonuclease-generated fragments of cellular DNA revealed identical patterns in the integration sites and internal recognition sites of MuMTV proviral equivalents in the tumor cells and normal organs of C57Bl/10 strain mice. The restriction DNA fragments of C57Bl/10-associated MuMTV proviral DNA are closely related to those of the Balb/c-associated MuMTV. These results indicate the endogenous origin of MuMTV produced in the hormonally stimulated cultures of DMBA-prolactin-estradiol-induced C57Bl/10 mammary tumors. (Author abstract) (35 Refs)

33. MOUSE MAMMARY TUMOR VIRUS DNA SEQUENCES IN TUMORIGENIC AND NONTUMORIGENIC CELLS FROM A MAMMARY ADENOCARCINOMA.

Morris VL, Gray DA, Jones RF, Chan EC, McGrath CM
Dept. Microbiology and Immunology, Univ. Western Ontario, London, Ontario N6A 5C 1, Canada
Virology; 118(1):117-127 1982

If BALB/c mice are foster nursed on C3H mice, which contain milk-borne mouse mammary tumor virus (MMTV) associated with a high mammary tumor incidence, their tumor incidence rises from less than 1 to 89.5% by 12 mo; in addition, there is an increase in the integrated MMTV DNA sequences in these mammary tumors. To investigate if all or only a few of these added MMTV DNA sequences are associated with tumorigenicity, we separated tumorigenic and nontumorigenic epithelial cells from a single mammary adenocarcinoma using primary methocel cultures. The MMTV DNA sequences from these cultures were then examined using restriction endonuclease analysis. Our results indicated that the methocel colonies isolated from a single mammary adenocarcinoma were heterogeneous with respect to their integrated MMTV DNA sequences. We also found that not all newly acquired MMTV DNA copies were associated with tumorigenicity. In addition, in one tumorigenic subline we detected only a single integrated copy. Our data thus suggest that the site of integration is more important than the total number of integrated MMTV DNA sequences. Furthermore, tumorigenicity did not seem to be linked to continued transcription of high levels of a major portion of the MMTV genome. (Author abstract) (28 Refs)

34. MOBILITY OF ENDOGENOUS ECOTROPIC MURINE LEUKEMIA VIRAL GENOMES WITHIN MOUSE CHROMOSOMAL DNA AND INTEGRATION OF A MINK CELL FOCUS-FORMING VIRUS-TYPE RECOMBINANT PROVIRUS IN THE GERM LINE.

Quint W, Van der Putten H, Janssen F, Berns A
Lab. Biochemistry, Univ. Nijmegen, 6525 EZ Nijmegen, The Netherlands
J Virol; 41(3):901-908 1982

Characterization of endogenous ecotropic Akv proviruses in DNA of low and high leukemic mouse strains revealed the presence of one to six copies of the Akv genome per haploid genome equivalent integrated in the germ line. Low leukemic strains analyzed so far contained only one complete copy of the Akv proviral DNA. The site of integration varied among strains, although genetically related strains often carried the Akv proviral gene in the same chromosomal site. The different substrains of the AKR mouse displayed the presence of variable numbers (2 to 6) of Akv genomes. In all substrains one Akv genome was present in an identical chromosomal site; this locus probably comprised the progenitor genome. Closely related substrains had several Akv proviral DNAs integrated in common sites. The accumulation of Akv genomes in the germ line of the AKR/FuRdA strain is likely the result of independent integration events, since backcross studies with the Akv-negative 129 strain showed random segregation of all six proviral loci. The AKR/Cnb strain carried a recombinant provirus in the germ line. This provirus resembled in structure the AKR mink cell focus-forming viruses, which are generated by somatic recombination during leukemogenesis. Therefore, the germline amplication of Akv proviral DNAs occurs most likely through infection of embryonic cells by circulating virus. (Author abstract) (28 Refs)

35. AMPLIFICATION AND REARRANGEMENT OF ONC GENES IN MAMMALIAN SPECIES.

Chattopadhyay SK, Chang EH, Lander MR, Ellis RW, Scolnick EM, Lowy DR
Dermatology Branch, NCI, NIH, Bethesda, MD, 20205
Nature; 296(5855):361-363 1982

Amplification and rearrangement of transforming (onc) genes was investigated in mammalian species. Sequences related to each onc have been identified in all vertebrate species tested, usually one per haploid genome. The rat has at least two different genes homologous to the onc of the rat-derived Harvey murine sarcoma virus (Ha-MuSV). Evidence for the relatively recent amplification of the genes was found by screening genomic DNA from several rodent sequences related to the onc of Ha-MuSV and to the closely related onc of Kirsten (Ki) MuSV. The ras sequences in rodent DNA were studied by electrophoresis and hybridization to a probe derived either from the v-Ha-ras (the onc gene of Ha-MuSV) or v-Ki-ras (the onc gene of Kirsten MuSV). The pattern of c-ras frag-

ments was distinctive for the DNAs from mink, rat, and other Mus species, while the v-Ha-ras probe detected the same c-ras fragments in all M musculus, M castaneus and M poschiavinus. In contrast to the relatively few c-ras fragments in other species, the DNA of a M pahari cell line contained many fragments, representing at least 10 copies per haploid genome. Amplification in M pahari apparently consists of c-Ha-ras sequences; such an occurrence does not seem to be the result of in vitro propagation of the cell line. Genomic DNA from Chinese hamster ovary cells digested with EcoRI contained at least eight fragments which hybridized to the v-Ki-ras probe, while Syrian hamster DNA contained only one such fragment. Possible mechanisms leading to amplification of the ras genes are discussed. (30 Refs)

36. ISOLATION AND CHARACTERIZATION OF A HUMAN LOCUS HOMOLOGOUS TO THE TRANSFORMING GENE (V-FES) OF FELINE SARCOMA VIRUS.

Trus MD, Sodroski JG, Haseltine WA
Lab. Biochemical Pharmacology, Sidney Farber Cancer Inst., Boston, MA, 02115
J Biol Chem; 257(6):2730-2733 1982

A single locus (designated c-fes) in the human genome which exhibits homology to the transformation-specific onc gene (v-fes) of Snyder-Theilen feline sarcoma virus was identified by the Southern blot technique. Recombinant clones containing 16-to 18-kilobase inserts of human DNA including the c-fes locus were constructed. Restriction endonuclease mapping of these clones verified their identity with native human c-fes and demonstrated the presence of at least two sequences in human c-fes interrupt ing v-fes-homologous regions. The v-fes-homologous locus in the human genome spans about 4 kilobases. The 5'-3' orientation of the c-fes clones with respect to feline sarcoma virus proviral DNA was determined. The region of the human genome that is homologous to v-fes is proximal to the highly reiterated human Alu sequence but not to the highly reiterated human alphoid sequence. (Author abstract) (39 Refs)

ANTIVIRALS

37. EFFECT OF INTERFERON ON THE EXOGENOUS FRIEND MURINE LEUKEMIA VIRUS INFECTION.

Riggin CH, Pitha PM
(c/o Pitha), Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21218
Virology; 118(1):202-213 1982

Interferon treatment (600 U/ml) of NIH/3T3 cells induced greater than 90% reduction in the de novo production of Friend MuLV when measured 24 hr postinfection. Early events in viral replication such as the synthesis of proviral DNA and its subsequent integration into the cell genome were not inhibited by interferon treatment indicating that the suppression of virus production by interferon appears to occur after synthesis of proviral DNA. Analysis of viral RNA species present in controls and interferon-treated cells 24 hr after infection show that the same RNA species were present in the presence and absence of interferon. Synthesis of viral polypeptides was reduced but not blocked in interferon-treated cells when measured within 24 hr after infection while processing of gag precursor, Pr65gag, and glycoprotein precursor, gPr85env, to viral proteins was not altered. Phosphorylation of viral protein p12 but not that of the precursor, Pr65gag, was inhibited in newly infected interferon-treated cells. In contrast to the first replicative cycle, interferon did not inhibit synthesis of viral proteins, and phosphorylation of p12 in those cells chronically infected with F-MuLV. (Author abstract) (32 Refs)

38. INHIBITORY EFFECTS OF VARIOUS 5-HALOGENATED DERIVATIVES OF 1-BETA-D-ARABINOFURANOSYLURACIL 5'-TRIPHOSPHATE ON DNA POLYMERASES FROM MURINE CELLS AND ONCORNAVIRUS: SUBSTITUENT EFFECTS ON INHIBITORY ACTION.

Ono K, Ogasawara M, Ohashi A, Matsukage A, Takahashi T, Nakayama C, Saneyoshi M
Lab. Viral Oncology, Aichi Cancer Center Res. Inst., Chikusa-ku, Nagoya 464, Japan
Biochemistry; 21(5):1019-1024 1982

The effects of four 5-halogenated derivatives of 1-beta-D-arabinofuranosyluracil 5'-triphosphate (aUTP) on the activity of DNA polymerases from mouse cells and oncornavirus were examined in comparison with that of the mother compound, aUTP, and also with those of the sister compounds, 5-alkylated derivatives of aUTP. The newly synthesized and tested compounds in this study were 1-beta-D-arabinofuranosyl-5-fluorouracil 5'-triphosphate (aFUTP), 1-beta-D-arabinofuranosyl-5-chlorouracil 5'-triphosphate (aClUTP), 1-beta-D-arabinofuranosyl-5-bromouracil 5'-triphosphate (aBrUTP), and 1-beta-D-a rabinofuranosyl-5-iodouracil 5'-triphosphate (aIUTP). The results are summarized. Relationships between the chemical structures of the analogues and their inhibitory effects are discussed according to inductive, hydrophobic, and steric characteristics of the substituents on the 5 position of the uracil nucleus. It was concluded that both steric and hydrophobic effects of the halogen substituents were important for the inhibition of all DNA polymerases and that the inductive effect of halogen atoms was clearly observed for DNA polymerase beta with activated DNA and RLV DNA polymerase with (rA)n.(dT)12-18. (22 Refs)

39. RESPONSE OF MURINE TUMOURS TO COMBINATIONS OF CCNU WITH MISONIDAZOLE AND AND OTHER RADIATION SENSITIZERS.

Siemann DW
Experimental Therapeutics Div., Univ. Rochester Cancer Center, Box 704, 601 Elmwood Ave., Rochester, NY, 14642
Br J Cancer; 45(2):272-281 1982

The effect of combinations of the conventional chemotherapeutic agent 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and nitroimidazole radiation sensitizers was evaluated in female C3H mice. Tumor response to single-agent or combination therapy was assessed in a tumor growth-delay assay. In the KHT sarcoma the simultaneous addition of misonidazole (MISO) was found to increase significantly the tumor growth delay resulting from CCNU treatment. The observed enhancement ratios (ER) increased with MISO dose, and ranged from 1.3 to 1.9 for sensitizer doses of 0.25-1.0 mg/g. The combination of CCNU and 1.0 or 0.5 mg/g MISO in the RIF-1 tumor or the MT-1 tumor produced ERs of approx 2.0 and 1.5 respectively. In the KHT sarcoma a series of other nitroimidazole sensitizers, including Ro-05-9963, SR-2555, SR-2508 and metronidazole (METRO), were also evaluated at equimolar doses (5 mmol/kg) in combination with a 20 mg/kg dose of CCNU. Unlike MISO, these compounds in general failed to en-

hance the CCNU cytotoxicity in this tumor model. However, SR-2508 did enhance the response of the RIF-1 tumor to large single doses of CCNU, though not as much as MISO. Normal-tissue toxicity was determined using peripheral white blood cell (WBC) counts 3 days after treatment. CCNU doses of 10-50 mg/kg given either alone or in simultaneous combination with 0.5 or 1.0 mg/g MISO were studied. WBC toxicity increased with CCNU dose, but the addition of MISO at either dose did not significantly enhance this normal-tissue toxicity. (Author abstract) (39 Refs)

U.S. GOVERNMENT PRINTING OFFICE : 1984—461-947/123

40. MONOAMINODICARBOXYLIC ACIDS AND THEIR AMIDES DURING TUMOR REGRESSION.

Balitskii KP, Sopotsinskaia EB, Lisniak IA
R. E. Kavetskii Inst. Problems in Oncology, Kiev, USSR
Ukr Biokhim Zh; 54(1):8-12 1982

Tissue distribution of monoaminodicarboxylic acids (MADCA) and their amides was studied in BALB/c mice bearing Moloney sarcoma. The 1.5-2 mo old mice inoculated with Moloney sarcoma v irus showed progressive tumor growth up to day 10, followed by a spontaneous tumor regression. The 10-12 day old mice did not show tumor regression. Progressive growth of Moloney sarcoma was associated with marked decrease of the MADCA levels in the liver, lungs, spleen, thymus, lymph nodes, and limbic system. Tumor regression was characterized by a relative stability or mild increase of MADCA in test organs. Administration of exogenous MADCA decreased the incidence of tumor-bearing mice, while administration of asparaginase enhanced the growth of Moloney sarcoma. (9 Refs) (In RUS)

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- **ANTIVIRALS** 33-38

VIRAL PROTEINS

1. STUDIES ON PROTEINS OF SIMIAN SARCOMA-ASSOCIATED VIRUS WITH DIFFERENT GROWTH HISTORY.

Warnaar SO, Huiskens-Van Der Meij C, Kool-Kofoed L, Mooren HW
Lab. Pathology, State Univ. Leiden, Wassenaarseweg 62, 2333 AL Leiden, The Netherlands
J Gen Virol; 59(part1):65-71 1982

Simian sarcoma-associated virus (SSaV) was repeatedly passaged on three human cell lines. The proteins of the progeny virus were analyzed for the presence of variant polypeptides. Occasionally, a few variant polypeptides were observed. One-dimensional peptide maps of the major virus protein p30 revealed no modifications after 25 cycles of infection on the three cell lines studied. The peptide map of Pr65gag of virus grown through 25 passages on a human chondrosarcoma cell line was slightly different from that of the virus stock before passaging. The relative amount of the virus protein p30 as compared to p18 and p16 (possibly the SSaV equivalents of p15E and p12E) was variable depending on the host cell. Virus grown on Daudi cells was relatively deficient in p18 and p16.

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These virus particles were morphologically altered and had a low infectivity. (Author abstract) (18 Refs)

2. P65 OF GAZDAR MURINE SARCOMA VIRUSES CONTAINS ANTIGENIC DETERMINANTS FROM ALL FOUR OF THE MURINE LEUKEMIA VIRUS (MULV) GAG POLYPEPTIDES (P15, P12, P30, AND P10) AND CAN BE CLEAVED IN VITRO BY THE MULV PROTEOLYTIC ACTIVITY.

Yoshinaka Y, Luftig RB
Dept. Microbiology, Mie Univ. Sch. Medicine, 174 Edobashi-2-Chome, Tsu, Mie 514, Japan
Virology; 118(2):380-388 1982

Thin-section electron micrographs of Gazdar murine sarcoma virus (Gz-MSV) particles showed that 100% of the particles possessed an immature morphology. Correspondingly, p65 (the major 65,000-dalton protein observed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis for Gz-MSV particles) possessed antigenic determinants from all four of the murine leukemia virus (MuLV) Pr65gag polypeptides - that is, p30, p15, p12, and p10. This result is in contrast to earlier observations which reported that p65 lacked antigenic determinants of MuLV p10. It is consistent with the recent finding of Maxwell and Arlinghaus (1981) that Gz-MSV p65, when cleaved in vitro gives rise to a polypeptide with the size and antigenic determinant of MuLV p10. Thus, we suggest that Gz-MSV p65 should be designated as Gz-MSV Pr65gag. We also found that Gz-MSV Pr65gag could be cleaved in vitro by using a partially purified proteolytic factor that had been derived from Moloney murine leukemia virus (M-MuLV) by Sephadex G-75 column chromatography. Protein bands were produced that migrated on gels and had the same antigenic determinants as the MuLV intermediates Pr40gag (p30, p10) and Pr27gag (p15, p12). Pr55gag (p15, p12, p30), a minor component, was also produced. Additional incubation of Gz-MSV Pr65gag led to a breakdown of the intermediate polyproteins into the four MuLV gag polypeptides p30, p10, p15, and p12. The final processing of Pr55gag and Pr40gag occurred more rapidly than that of Pr27gag. It thus seems that in vitro sequentially different processing events are involved in production of the four internal gag antigens from Gz-MSV Pr65gag. (Author abstract) (23 Refs)

3. MOLECULAR BIOLOGY CRITERIA FOR THE FUNCTION OF ROUS SARCOMA VIRUS GENE IN TRANSFORMED KHS RAT CELLS.

Kiselev FL, Galetskii SA, Mazurenko NN, Asanova AA, Zborovskaia IB, Topol' LZ
All-Union Oncology Res. Center, USSR Acad. Medical Sciences, Moscow, USSR
Mol Biol (Mosk); 16(1):66-76 1982

Integration, transcription and translation of Rous sarcoma virus (RSV) genome was studied in (non-virus-producing) transformed rat fibrosarcoma XC cells. About 10 copies of RSV genome were integrated into the host cell genome. Different types of viral RNAs, identified in the cytoplasmic fraction, had sedimentation values similar to those for RSV-specific RNAs in virus-producing chicken cells. These RNAs were translated in polysomes and directed the synthesis of gag, env and src proteins. Compared with chicken cells, the cleavage of gag precursor Pr 76gag was not observed in transformed XC cells. (30 Refs) (In RUS)

4. CHARACTERIZATION OF AN RNA-DIRECTED DNA POLYMERASE FROM MOUSE SPLEEN INFECTED WITH LEUKAEMIA VIRUS ACTIVATED DURING N-METHYL-N-NITROSO UREA-INDUCED LEUKAEMOGENESIS.

Drescher B, Ehm I, Fey F
Central Inst. Molecular Biology, Acad. Sciences of German Democratic Republic, DDR-115 Berlin-Buch, E. Germany
Acta Virol (Praha); 26(1/2):41-49 1982

An RNA-directed DNA polymerase was purified from mouse spleen infected with leukemia virus activated during N-methyl-N-nitroso urea- (MNU-) induced leukemogenesis. The enzyme was isolated from the microsomal fraction and purified by successive chromatography of Sephadex G-200 and phosphocellulose. Estimation of mol wt from the sedimentation rate of the purified enzyme in a sucrose gradient gave a value of 70,000. The enzyme had a pH optimum of 7.4, a KCl optimum of 50 mmol/l, an Mn++ optimum of 0.2 mmol/l, and a temperature optimum of 25 C, when (rA)n - (dT)10 was used as the template-primer. It preferred (rA)n - (dT)10 as the template-primer and transcribed (rC)n - (dG)12 and (OMeC)n - (dG)12. A comparison of the properties of this DNA polymerase with the enzyme purified from murine Type C retroviruses showed that the MNU-activated virus enzyme was both biochemically and biophysically indistinguishable from murine leukemia virus DNA polymerases. (Author abstract) (18 Refs)

5. GLYCOPROTEINS OF FRIEND MURINE LEUKEMIA VIRUS: SEPARATION AND NH2-TERMINAL AMINO ACID SEQUENCES OF GP69 AND GP71.

Linder D, Stirm S, Schneider J, Hunsmann G, Smythers G, Oroszlan S
(c/o Oroszlan) Biological Carcinogenesis Program, Frederick Cancer Res. Facility, NCI, Frederick, MD, 21701
J Virol; 42(1):352-355 1982

The NH2-terminal amino acid sequences (initial 23 residues) of Friend murine leukemia virus gp71 and gp69 were determined and found to be different but highly related. Friend murine leukemia virus gp71 differed from Rauscher murine leukemia virus gp70 in only one position. Friend murine leukemia virus gp69 showed approx 41% homology to these glycoproteins but lacked the glycosylation site (sequon) occurring at position 12 in Rauscher murine leukemia virus gp 70. (Author abstract) (17 Refs)

6. OLIGOSACCHARIDE MODIFICATIONS AND THE SITE OF PROCESSING OF GPR92ENV, THE PRECURSOR FOR THE VIRAL GLYCOPROTEINS OF ROUS SARCOMA VIRUS.

Bosch JV, Schwarz RT, Ziemiecki A, Friis RR
Institut fur Virologie, Justus-Liebig-Universitat Giessen, Fachbereich Humanmedizin, Frankfurter Strasse 107, D-6300 Giessen, W. Germany
Virology; 119(1):122-132 1982

We have studied the processing of the RSV glycoprotein precursor gPr92env to mature viral disulfide-linked gp85 and gp35 (VGP). The first aspect concerned the steps involved in the processing event. We could show that, in addition to proteolytic cleavage, the processing of gPr92env involves the conversion of oligosaccharides from the high-mannose type found in gPr92env to the complex, sialidated type found in VGP. Concerning the site of processing, we failed to demonstrate gPr92env in newly released virions but could find a small amount of gPr92env on the cell surface. It is thus conceivable,

but unlikely, that processing is taking place at the cell surface. gp85 and gp35 could be detected in low, somewhat variable amounts in cell lysates and we have shown that most of this is within the cell and not at the cell surface. We favor the possibility that processing of gPr92env is occurring at the usual sites of glycosylation, namely in the rough ER and Golgi complex, and that the surface gPr92env is a 'dead-end' product which does not become incorporated into virus. (Author abstract) (34 Refs)

7. IDENTIFICATION OF A PRECURSOR IN THE BIOSYNTHESIS OF THE P21 TRANSFORMING PROTEIN OF HARVEY MURINE SARCOMA VIRUS.

Shih TY, Weeks MO, Gruss P, Dhar R, Oroszlan S, Scolnick EM
Lab. Tumor Virus Genetics, NCI, Bethesda, MD, 20205
J Virol; 42(1):253-261 1982

The p21 transforming protein coded for by the v-ras gene of Harvey murine sarcoma virus (Ha-MuSV) migrates as a doublet band between 21,000 and 23,000 daltons during sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The lower band of the doublet is designated p21, and the upper band is designated pp21 since it comigrates with the phosphorylated form of p21. By pulse-labeling with [35S]methionine, we detected a p21 precursor, pro-p21, which migrated as if it was approx 1,000 daltons larger than p21. The precursor-product relationship was established by pulse-chase experiments with [35S]methionine in the presence of 100 ug of cycloheximide per ml, which inhibited all de novo protein biosynthesis. Within 4 h, pro-p21 was completely chased into p21, and during the next 24 h pp21 accumulated. Thus, formation of pp21 from p21 did not require de novo protein synthesis. By subcellular fractionation into cytosol and membrane fractions, we found that pro-p21 was synthesized in a non-membrane-bound state and that shortly after its complete synthesis, the p21 product was associated with the membrane fraction. By selective cleavage of p21 at a unique aspartic acid-proline residue with 70% formic acid or with Staphylococcus aureus V8 protease, we found that the intramolecular site of pro-p21 processing was located in the C-terminal portion of the pro-p21 molecule. The possibilities that the precursor was involved in the assembly of p21 into the plasma membrane and, alternatively, that the processing was a step in the activation of p21 biochemical activities are discussed. (Author abstract) (18 Refs)

8. CANDIDATE PRODUCT OF THE FBJ MURINE OSTEOSARCOMA VIRUS ONCOGENE: CHARACTERIZATION OF A 55,000-DALTON PHOSPHOPROTEIN.

Curran T, Teich NM
Imperial Cancer Res. Fund Lab., Lincoln's Inn Fields, London WC2A 3PX, England
J Virol; 42(1):114-122 1982

Sera from rats bearing tumors induced by inoculation of FBJ murine osteogenic sarcoma virus (FBJ-MSV) nonproducer rat cells precipitate two proteins with mol wt of 55,000 (p55) and 39,000 (p39) from FBJ-MSV-transformed cells. These proteins cannot be precipitated from uninfected cells or cells transformed by other strains of murine sarcoma virus, nor can they be precipitated by sera specific for the viral structural proteins. A methionine tryptic peptide mapping analysis showed that p55 and p39 have little or no homology and that they are not related to the helper virus gag and env gene products. p55 could also be detected among the in vitro translation products of 70S RNA from FBJ murine leukemia virus plus FJB-MSV virions but not among those from FBJ murine leukemia virus alone. This suggests that p55 is encoded by the FBJ-MSV genome, whereas p39, which was not detected among the in vitro translation products, may not be virus encoded. Another difference between p55 and p39 is that p55 is phosphorylated, with most of the phosphate on a serine residue(s), whereas p39 is phosphorylated to a much lesser extent, if at all. No protein kinase activity was associated with p55 and p39 immune complexes under standard conditions. Our data suggest that p55 is a strong candidate for the FBJ-MSV oncogene product. (Author abstract) (32 Refs)

9. A CELLULAR PROTEIN IS IMMUNOLOGICALLY CROSSREACTIVE WITH AND FUNCTIONALLY HOMOLOGOUS TO THE FUJINAMI SARCOMA VIRUS TRANSFORMING PROTEIN.

Mathey-Prevot B, Hanafusa H, Kawai S
Rockefeller Univ., New York, NY, 10021
Cell; 28(4):897-906 1982

We obtained a regressing-tumor antiserum specific for the unique sequence of the transforming protein P140 of Fujinami sarcoma virus by injecting Fischer rats with syngeneic embryo cells transformed with Fujinami sarcoma virus. This serum is capable of immunoprecipitating a protein of 98,000 daltons from cell extracts of normal, uninfected chicken bone marrow cells. This normal cellular protein (NCP98) was shown to be structurally related to P140, sharing the majority of 35S-methionine-labeled tryptic peptides with the viral gene product P140. NCP98 is a phosphoprotein in vivo, with an association in vitro protein kinase activity, capable of phosphorylating specifically at tyrosine residues of NCP98 itself and alpha-casein, an externally added substrate. This kinase activity is biochemically indistinguishable from the kinase activity is biochemically indistinguishable from the kinase activity associated with P140 by all criteria tested. Moreover, in vitro-phosphorylated NCP98 and P140 shared the same phosphopeptides. The expression of NCP98 is tissue-specific. It is readily detectable in bone marrow cells and detectable to a lesser extent in liver and lung cells from 6-18 day old chickens. (Author abstract) (44 Refs)

10. AVIAN SARCOMA VIRUS UR2 ENCODES A TRANSFORMING PROTEIN WHICH IS ASSOCIATED WITH A UNIQUE PROTEIN KINASE ACTIVITY.

Feldman RA, Wang LH, Hanafusa H, Balduzzi PC
Rockefeller Univ., New York, NY, 10021
J Virol; 42(1):228-236 1982

UR2 is a newly characterized avian sarcoma virus whose genome contains a unique sequence that is not related to the sequences of other avian sarcoma virus transforming genes thus far identified. This unique sequence, termed ros, is fused to part of the viral gag gene. The product of the fused gag-ros gene of UR2 is a protein of 68,000 daltons (P68) immunoprecipitable by antiserum against viral gag proteins. In vitro translation of viral RNA and in vivo pulse-chase experiments showed that P68 is not synthesized as a larger precursor and that it is the only protein product encoded in the UR2 genome, suggesting that it is involved in cell transformation by UR2. In vivo, P68 was phosphorylated at both serine and tyrosine residues. Immunoprecipitates of P68 with anti-gag antisera had a cyclic nucleotide-independent protein kinase activity that phosphorylated P68, rabbit immunoglobulin G in the immune complex, and alpha-casein. The phosphorylation by P68 was specific to tyrosine of the substrate proteins. P68 was phosphorylated in vitro at only one tyrosine site, and the tryptic phosphopeptide

of in vitro-labeled P68 was different from those of Fujinami sarcoma virus P140 and avian sarcoma virus Y73-P90. A comparison of the protein kinases encoded by UR2, Rous sarcoma virus Fujinami sarcoma virus, and avian sarcoma virus Y73 revealed that UR2-P68 protein kinase is distinct from the protein kinases encoded by those viruses by several criteria. Our results suggest that several different protein kinases encoded by viral transforming genes have the same functional specificity and cause essentially the same cellular alterations. (Author abstract) (32 Refs)

11. HALF-LIFE OF THE ROUS SARCOMA VIRUS TRANSFORMING PROTEIN PP66SRC AND ITS ASSOCIATED KINASE ACTIVITY.

Ziemiecki A, Friis RR, Bauer H
Institut fur Virologie, Fachbereich Humanmedizin, Justus-Liebig-Universitat, 6300 Giessen, W. Germany
Mol Cell Biol; 2(4):355-360 1982

Chick embryo cells infected with the Prague A strain of Rous sarcoma virus and several transformation-defective, temperature-sensitive mutants were pulse-labeled with [35S]methionine; the half-lives of metabolically labeled pp60src and the associated protein kinase activity were determined. Irrespective of the temperature at which the cells were grown, pp60src had a half-life of approx 60 min in Wild-type and mutant-infected cells. Treatment with cycloheximide, emetine, cordycepin, or puromycin significantly prolonged the half-life of the pp60src-associated protein kinase activity and the half-life of metabolically labeled pp60src. The apparent contradiction between the half-lives of the kinase activity and the labeled pp60src protein was resolved by the observation that treatment of cells with inhibitors of protein synthesis stabilized pp60src, resulting in a greatly extended half-life. Since protein synthesis inhibitors also extended the half-life of the gag precursor polypeptide, Pr76, a host factor(s) may be necessary for efficient intracellular processing of the polypeptide to the gag proteins. (30 Refs)

12. CHANGES IN AMINO-TERMINAL SEQUENCES OF PP60SRC LEAD TO DECREASED MEMBRANE ASSOCIATION AND DECREASED IN VIVO TUMORIGENICITY.

Krueger JG, Garber EA, Goldberg AR, Hanafusa H
Rockefeller Univ., New York, NY, 10021
Cell; 28(4):889-896 1982

We have suggested previously that the amino-terminal 8 kilodaltons of pp60src may serve as a structural hydrophobic domain through which pp60src attaches to plasma membranes. Two isolates of recovered avian sarcoma viruses (rASVs), 1702 and 157, encode pp60src proteins that have alterations in this amino-terminal region. The rASV 1702 src protein (56 kilodaltons) and the 157 src protein (62.5 kilodaltons) show altered membrane association, and fractionate largely as soluble, cytoplasmic proteins in aqueous buffers, in contrast with the membrane association of more than 80% of the src protein of standard avian sarcoma virus under the identical fractionation procedure. Plasma membranes purified from cells transformed by these rASVs contain less than 10% of the amount of pp60src found in membranes purified from cells transformed by Rous sarcoma virus or control rASVs. The altered membrane association of these src proteins had little or no effect on the properties of chick embryo fibroblasts transformed in monolayer culture. In contrast, rASV 1702 showed reduced in vivo tumorigenicity compared with Rous sarcoma virus or with other rASVs that encode membrane-associated src proteins. Rous sarcoma virus-induced tumors are malignant, poorly differentiated sarcomas that are lethal to their hosts. rASV 1702 induces a benign, differentiated sarcoma that regresses and is not lethal to its hosts. These data support the role of amino-terminal sequences in the membrane association of pp60src, and suggest that the amino terminus of pp60src, have a critical role in the promotion of in vivo tumorigenicity. (Author abstract) (14 Refs)

13. IDENTIFICATION OF A FISH PROTEIN ASSOCIATED WITH A KINASE ACTIVITY AND RELATED TO THE ROUS SARCOMA VIRUS TRANSFORMING PROTEIN.

Barnekow A, Schartl M, Anders F, Bauer H
Institut fur Virologie, Fachbereich Humanmedizin, Justus-Liebig-Universitat Giessen, Frankfurter Strasse 107, D-6300 Giessen, W. Germany
Cancer Res; 42(6):2429-2433 1982

Cells of all vertebrates thus far investigated contain a cellular src gene, which appears to be a highly conserved homolog of the Rous sarcoma virus oncogene src. Similar to the Rous sarcoma virus oncogene src, this endogenous cellular src gene codes for a Mr 60,000 phosphoprotein (pp60c-src) which is associated with a kinase activity. This kinase specifically phosphorylates tyrosine residues of proteins. We have systematically looked for pp60c-src in specific strains of certain teleostean fish, including the genera Xiphophorus and Girardinus. These strains are known to contain chromosomal oncogenes causing neoplastic transformation in certain mutants and hybrids. Since the pp60c-src found in other species has been shown to be antigenically related to the viral src gene product, a phosphoprotein with a molecular weight of 60,000 (pp60r-src), we have used tumor-bearing rabbit serum which recognizes Rous sarcoma virus pp60src to detect such a related protein in fish. Tumor-bearing rabbit serum precipitated two proteins with molecular weights of 60,000 and 52,000. The Mr 60,000 phosphoprotein was associated with a protein kinase activity that phosphorylates tyrosine residues of the immunoprecipitating immunoglobulin G heavy chain (Mr 53,000). The antigenic relationship of this fish protein to viral pp60src was further documented by an immune competition assay. We conclude, therefore, that the fish Mr 60,000 phosphoprotein is a pp60c-src encoded for by a fish cellular src gene that corresponds to the viral src oncogene. The Mr 52,000 phosphoprotein is probably a proteolytic degradation product of pp60c-src. All animals of the various strains of the fish investigated in this study showed kinase activity that was reactive upon immunoprecipitation with tumor-bearing rabbit serum, indicating that the pp60c-src protein is a normal constituent of the fish. Measurable quantitative differences were revealed between the various strains as well as in different tissues. Whereas brain and melanoma tissue showed a high kinase activity when compared to skin, liver, spleen, and testes, muscle tissue displayed no significant activity. (Author abstract) (27 Refs)

14. CONSTRUCTION OF PLASMIDS FOR EXPRESSION OF ROUS SARCOMA VIRUS TRANSFORMING PROTEIN, P60SRC, IN ESCHERICHIA COLI.

Gilmer TM, Parsons JT, Erikson RL
Dept. Pathology, Univ. Colorado Sch. Medicine, Denver, CO, 80262
Proc Natl Acad Sci USA; 79(7):2152-2156 1982

We have constructed plasmids that direct the synthesis of the Rous sarcoma virus transforming gene (src) product (p60src) in Escherichia coli. A 203-base-pair lac promoter-operator DNA encoding the first eight amino acids or beta-galac-

tosidase was ligated to the 5' end of the src gene from the Prague A strain of Rous sarcoma virus (PrA-RSV) which had been cloned in pBR325. Antiserum, from a tumor-bearing rabbit, directed against pp60src was used to screen bacteria containing the recombinant plasmid for a protein of approx 60,000 daltons, and several colonies producing a protein immunologically related to pp60src were detected. Partial proteolytic cleavage analysis revealed that the src-related protein produced in bacteria is structurally similar to pp60src immunoprecipitated from PrA-RSV-infected chicken cells. Partially purified src protein from E coli can be phosphorylated in vitro by the catalytic subunit of cAMP-dependent protein kinase. Tryptic phosphopeptide analysis demonstrated that the catalytic subunit phosphorylated a serine-containing tryptic peptide in the bacterial src protein that comigrated with the phosphoserine-containing tryptic peptide of pp60src immunoprecipitated from 32P-labeled PrA-RSV-infected chicken cells. (Author abstract) (31 Refs)

15. STUDIES ON THE STRUCTURE OF AVIAN MYELOBLASTOSIS VIRUS (AMV) RNA. I. FACTORS AFFECTING ELECTRON MICROSCOPIC VISUALIZATION OF AMV RNA.

Stokrova J, Korb J, Riman J
Inst. Molecular Genetics, Czechoslovak Acad. Sciences, 160 20 Prague, Czechoslovakia
Acta Virol (Praha); 26(1/2):1-11 1982

Factors affecting the visualization of single-stranded (ss) avian myeloblastosis virus (AMV) RNA by the basic protein film technique under strong denaturation conditions were studied. The basic parameters of the electron microscopic picture of ss RNA, involving the visibility, concentration and contour lengths of molecules, were found to be significantly dependent on the (1) quality of denaturing components used for full extension of AMV RNA molecules and (2) spreading and formation of the surface film. Under the conditions used, the presence of artificial secondary structures on AMV RNA molecules was dependent on batches of formamide and urea in the denaturing mixture. The spreading and formation of the surface film were affected by hydrophilicity of glass spreading ramps, speed of spreading, amount of spreading solution, amount of protein in the hyperphase, type of cytochrome c and the presence of substances influencing surface tension. The optimal conditions and spreading arrangement for visualization of ss AMV RNA are described. (Author abstract) (34 Refs)

16. STUDIES ON THE STRUCTURE OF AVIAN MYELOBLASTOSIS VIRUS (AMV) RNA. II. INTEGRITY OF THE RNA IN DEPENDENCE ON RNA ISOLATION AND VIRUS PROPAGATION.

Stokrova J, Korb J, Riman J
Inst. Molecular Genetics, Czechoslovak Acad. Sciences, 160 20 Prague, Czechoslovakia
Acta Virol (Praha); 26(1/2):12-24 1982

While sedimentation analysis revealed no basic changes in the sedimentation characteristics of 60-70 S avian myeloblastosis virus (AMV) RNA isolated under different conditions from two sources, electron microscopy exhibited differences in the length distributions of this RNA. Based on the length distribution profiles, phenol extraction showed a higher degradation effect in comparison with direct gradient centrifugation of viral lysates. Proteinase K appeared to be a more suitable nuclease inhibitor than diethylpyrocarbonate during the isolation of AMV RNA. Fundamental differences between length distributions of 60-70 S RNA from plasma AMV and that from AMV propagated in cultured leukemic myeloblasts were found. Precipitation with ethanol had no effect on the length distribution of AMV RNA. Nor did storage of leukemic chicken plasma at-70 C and one thawing substantially influence the lengths of AMV RNA. The heterogeneous length distributions of 60-70 S AMV RNA molecules with preferential size-classes were independent of virus propagation and RNA isolation. The results support the idea of preferential splitting of AMV RNA at specific sites. (Author abstract) (36 Refs)

17. STUDIES ON THE STRUCTURE OF AVIAN MYELOBLASTOSIS VIRUS (AMV) RNA. III. ELECTRON MICROSCOPIC DEFINITION OF SECONDARY STRUCTURE.

Stokrova J, Korb J, Riman J
Inst. Molecular Genetics, Czechoslovak Acad. Sciences, 160 20 Prague, Czechoslovakia
Acta Virol (Praha); 26(1/2):25-32 1982

The secondary structure of avian myeloblastosis virus (AMV) RNA was characterized by electron microscopy under moderately denaturing spreading conditions. Under denaturation by aqueous 44% formamide or 77% formamide in the presence of salts, partly stretched RNA molecules with measurable double-stranded regions were observed. This approach allowed the localization from 5 to 11 regions of preserved secondary structure on AMV RNA molecules. Topographic analysis revealed a nonrandom occurrence of stable secondary structures in several prevalent regions. These regions with higher secondary structure stability revealed certain similarity to hairpin structures localized by electron microscopy on Rous sarcoma virus RNA or to highly structured regions found on this RNA by T1 ribonuclease oligonucleotide analysis. (Author abstract) (22 Refs)

18. SUBGENOMIC MRNA IN OK10 DEFECTIVE LEUKEMIA VIRUS-TRANSFORMED CELLS.

Saule S, Sergeant A, Torpier G, Raes MB, Pfeifer S, Stehelin D
INSERM U186, Oncologie Moleculaire, Institut Pasteur, 59019 Lille Cedex, France
J Virol; 42(1):71-82 1982

OK10, a defective leukemia virus, is produced as a defective particle by so-called nonproducer transformed quail fibroblasts. OK10 defective viral particles contain an 8-kilobases (kb)-long genomic RNA, lack any detectable reverse transcriptase activity, and are not infectious. We studied the genetic content of OK10 RNA extracted from both virions and infected cells. About 77% of the OK10 8.0-kb RNA was related to avian leukosis viruses in the three structural genes gag, pol, and env, as well as in the c region. The remainder of the OK10 genome-encoding capacity was homologous to the MC29-specific transforming sequence myc(m) and has been named myc(o). Heteroduplex molecules formed between the viral OK10 8.0-kb RNA and the 6.8-kb SacI DNA fragment of the Prague A strain of Rous sarcoma virus indicated that the myc(o) sequence formed a continuous RNA stretch of 1.4 to 1.6 kb long between Delta-pol and Deltaenv. We also examined the myc(o)-containing mRNA's transcribed in OK10-transformed cells. In OK10-transformed bone marrow cells (OK10 BM), a stable bone marrow-derived cell line producing OK10, the myc(o) sequence was found in four RNA species of 11.0, 8.0, 7.0, and 3.6 kb. Again, the genetic content of these mRNA's indicated that (i) the 3.6-kb species could be spliced out of the 8.0-kb-genome size mRNA and (ii) the 11.0-kb-long mRNA could represent a read-through of the OK10 provirus, the corre-

sponding maturation product being, then, a 7.0-kb mRNA. In conclusion, whereas the myc sequences have been generally thought to be expressed through a gag-onc fusion protein, as for MC29 and CMII viruses, our experiments indicate that they could also be expressed as a non-gag-related product made from a subgenomic mRNA in the OK10 transformed cells. (34 Refs)

19. DIFFERENTIAL EXPRESSION OF THE AMV GENE IN HUMAN HEMATOPOIETIC CELLS.

Westin EH, Gallo RC, Arya SK, Eva A, Souza LM, Baluda MA, Aaronson SA, Wong-Staal F
Lab. Tumor Cell Biology, Div. Cancer Treatment, NCI, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 79(7):2194-2198 1982

Total cellular RNAs from a variety of fresh and culture-derived human hematopoietic neoplastic cell types at various stages of differentiation and human sarcoma, carcinoma, melanoma, and glioblastoma cell lines were enriched for poly(A)-containing sequences, fractionated by gel electrophoresis, and blot hybridized to a cloned DNA probe containing the transforming sequences (v-amv) of avian myeloblastosis virus (AMV), a virus known to cause myeloid leukemias in chickens. Expression of RNA sequences homologous to AMV was detected in all immature myeloid and lymphoid T cells in addition to single erythroid cell line examined, but not in mature T cells or in B cells, including lymphoblast cell lines derived from patients with Burkitt lymphoma. In addition, induction of the cell line HL60, a promyelocytic leukemia line, to differentiate with dimethyl sulfoxide or retinoic acid resulted in a reduction of the level of expression of the human cellular gene c-amv homologous to v-amv. There was no detectable c-amv mRNA in any of the solid tumor cell lines examined. Thus, expression of the human c-amv gene could be correlated with the stage of differentiation of different hematopoietic cell types determines by morphologic and marker studies. Expression of c-amv could not be correlated with the extent of methylation in HL60 and in HL60 induced to differentiate with dimethyl sulfoxide. (Author abstract) (48 Refs)

20. EXPRESSION OF CELLULAR HOMOLOGUES OF RETROVIRAL ONC GENES IN HUMAN HEMATOPOIETIC CELLS.

Westin EH, Wong-Staal F, Gelmann EP, Favera RD, Papas TS, Lautenberger JA, Eva A, Reddy EP, Tronick SR, Aaronson SA, Gallo RC
Lab. Tumor Cell Biology, NCI, NIH, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 79(8,part1):2490-2494 1982

Total cellular poly(A)-enriched RNA from a variety of fresh human leukemic blood cells and hematopoietic cell lines was analyzed for homology with molecularly cloned DNA probes containing the ONC sequence of Abelson murine leukemia virus (Ab-MuLV), Harvey murine sarcoma virus (Ha-MuSV), simian sarcoma virus (SSV), and avian myelocytomatosis virus strain MC29. Results with the fresh blood cells paralleled those obtained with the cell lines. With Ab-MuLV and Ha-MuSV, multiple RNA bands were visualized in all cell types examined without significant variation in the relative intensities of the bands. When SSV was used as the probe, expression of related onc sequences was absent in all of the hematopoietic cell types examined except for one neoplastic T-cell line (HUT 102), which produces the human T-cell leukemia (lymphoma) retrovirus HTLV. In this cell line, a single band (4.2 kilobases) was observed. With MC29 as the probe, a single band of 2.7 kilobases was visualized in all cell types examined with only a one- to two-fold variation in intensity of hybridization. An exception was the promyelocytic cell line, HL60, which expressed approximately 10-fold more MC29-related onc sequences. With induction of differentiation of HL60 with either dimethyl sulfoxide or retinoic acid, a marked diminution in the amount of the MC29-related, but not the Ab-MuLV-related, onc message was observed. (Author abstract) (62 Refs)

21. DETECTION OF BOVINE LEUKAEMIA VIRUS RNA SEQUENCES IN NON-CULTIVATED PERIPHERAL LYMPHOCYTES BY IN SITU HYBRIDIZATION WITH 3H-LABELLED VIRAL CDNA.

Notzel U, Drescher B, Rosenthal S
Central Inst. Molecular Biology, Acad. Sciences of German Democratic Republic, DDR-1115 Berlin-Buch, E. Germany
Acta Virol (Praha); 26(1/2):33-40 1982

Bovine leukemia virus (BVL) in circulating lymphatic cells of the blood is repressed and has to be activated for its expression. After in situ hybridization of BLV-3HcDNA with a specific activity of 3.9 x 10(7) dpm/ug to noncultivated blood cells from five leukotic cattle, in four of these animals from 0.96% to 1.5% lymphocytes were found to contain BLV-specific RNA sequences. Non-hybridized lymphocytes as well as calf thymus cells, used as controls, gave only 0.47% and 0.39% labeled cells, respectively. After short-term cultivation of white blood cells from these leukotic animals the percentage of labeled cells increased. During the short-term cultivation the number of hybridizing cells from one and the same animal and from all animals, when compared with each other, varied to a high extent. The highest values of BLV-RNA sequences-containing cells from all five animals at different cultivation times were within the range of from 2.4 to 16.2%. (Author abstract) (27 Refs)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

22. TRANSFORMING GENES OF CHICKEN BURSAL LYMPHOMAS.

Cooper GM
Dept. Pathology, Harvard Medical Sch., Boston, MA, 02115
J Cell Physiol; Suppl1:209-212 1982

Transforming genes (TG) in chicken bursal lymphomas (BL), isolation of such TG by molecular cloning, and the specificity of TG activated in neoplasms of different types of differentiated cells are discussed. BL are induced in chickens by infection with lymphoid leukosis viruses (LLV). Transfection assays of DNA of LLV-induced BL indicated that these neoplasms contained TG that induced transformation of NIH 3T3 mouse cells with efficiencies of approx 0.1 transformants/ug DNA. No viral sequences were detected in NIH cells transformed by LLV-induced lymphoma DNA using probes either representative of the entire LLV genome or specific for the LLV long terinmal redundancy, which contains the viral transcriptional promoter. TG of BL detected by transfection were linked to viral DNA, indicating that lymphomagenesis by LLV involved indirect activation of cellular TG at some stage of the disease process. DNA of NIH cell transformed by BL cell line RP-9 DNA was subjected to partial digestion with four-base recognition restriction endonuclease Mbo I and cloned in lambda Charon 30 to produce a library of approx 200,000 phage. Restriction fragments of normal chicken DNA were de-

tected in blot hybridization with lambda ChBlym-1 probe indicating that the lambda ChBlym-1 TG is homologous to normal chicken sequences. Analysis of TG of B and T cells neoplasms of mouse and human origin further indicates that specific TG are activated in neoplasms representative of specific stages of lymphocyte differentiation. Results thus indicate that specific TG are activated in neoplasms of particular differentiated cell types and suggests the hypothesis that neoplasia may result from abnormal expression of genes that are normally involved in differentiation-specific growth control. (15 Refs)

23. STRUCTURE OF A REPLICATION INTERMEDIATE IN THE SYNTHESIS OF ROUS SARCOMA VIRUS DNA IN VIVO.

Swanstrom R, Bishop JM, Varmus HE

Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143

J Virol; 42(1):337-341 1982

Intermediates in the synthesis of Rous sarcoma virus DNA in vivo contain a short second strand of DNA (plus strong-stop DNA) synthesized by using the region near the 5' end of the first (minus) strand of DNA as the template. In this report, we show that the 3' end of plus strong-stop DNA is extended about 15 to 20 nucleotides beyond the 5' end of the minus-strand DNA template, probably copying a portion of the tRNATrp molecule that serves as primer for synthesis of the minus strand of DNA. The extra sequences present in plus strong-stop DNA may play a central role in the generation of the long terminal repeat present in mature forms of viral DNA. (Author abstract) (26 Refs)

24. SYNTHESIS OF MURINE LEUKEMIA VIRAL DNA IN VITRO: EVIDENCE FOR PLUS-STRAND DNA SYNTHESIS AT BOTH ENDS OF THE GENOME.

DesGroseillers L, Rassart E, Zollinger M, Jolicoeur P

Institut de Recherches Cliniques de Montreal, Universite de Montreal, Montreal, Quebec, H2W 1R7, Canada

J Virol; 42(1):326-330 1982

We studied the synthesis of B-tropic murine leukemia viral DNA in vitro by detergent-disrupted virions. The reaction products (detected by the Southern transfer technique) included full-length, infectious, double-stranded DNA and several subgenomic fragments. Restriction endonuclease analysis and hybridization with specific probes revealed two classes of subgenomic fragments: some were derived from the right end of the genome, and some were derived from the left end. Most of the fragments harbored one long terminal repeat copy at their ends, suggesting that they were initiated correctly. S1 nuclease and restriction endonuclease treatments of these fragments indicated that a single-stranded gap was present near the first initiation site of plus strong-stop DNA. The treatments also suggested the presence of a second initiation site flanked by a single-stranded gap 0.9 kilobase pairs from the right end of the genome. Our data clearly show that plus-strand DNA is synthesized at both ends of the genome, by using plus strong stop as the first initiation site and additional initiation sites. (Author abstract) (24 Refs)

25. RESTRICTION ENDONUCLEASE AND NUCLEOTIDE SEQUENCE ANALYSES OF MOLECULARLY CLONED UNINTEGRATED AVIAN TUMOR VIRUS DNA: STRUCTURE OF LARGE TERMINAL REPEATS IN CIRCLE JUNCTIONS.

Katz RA, Omer CA, Weis JH, Mitsialis A, Faras AJ, Guntaka RV (c/o Guntaka) Dept. Microbiology, Coll. Physicians and Surgeons, Columbia Univ., New York, NY, 10032

J Virol; 42(1):346-351 1982

Avian tumor virus supercoiled DNA was isolated from infected quail tumor cells and molecularly cloned in pBR322. Four different recombinant clones denoted pATV-6, pATV-7, pATV-8, and pATV-9 were characterized in detail by restriction endonuclease mappings and by DNA sequencing. The results of these studies indicate that (i) the two large terminal repeats (LTRs) present in pATV-6 are different sizes, (ii) pATV-8 and pATV-9 contain only one LTR, (iii) pATV-7 contains an inversion of 0.6 kilobase in the env gene and a deletion of the U3 region and the src gene, and (iv) the src gene is deleted in pATV-6 and pATV-9. Circle formation from linear molecules was also examined in several of the clones by DNA sequencing through the circle joint. pATV-6 is an example of one class of circular molecules and contains a partially repeated LTR similar to that reported by Ju and Skalka. A second class of circles was exemplified by pATV-8 and pATV-9, which contain a single copy of the LTR with no base changes or deletions. This is in contrast to a class of circles containing a complete double LTR structure described by Swanstrom et al and suggests that circles containing a single intact LTR may be formed by a homologous recombinational event in which an entire LTR or complementary regions from both LTRs are removed from the linear DNA molecule during circularization. (Author abstract) (24 Refs)

26. GENOME OF RETICULOENDOTHELIOSIS VIRUS: CHARACTERIZATION BY USE OF CLONED PROVIRAL DNA.

Rice NR, Hiebsch RR, Gonda MA, Bose HR, Gilden RV

Biological Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD, 21701

J Virol; 42(1):237-252 1982

Reticuloendotheliosis virus is an avian Type C retrovirus that is capable of transforming fibroblasts and hematopoietic cells both in vivo and in vitro. Studies have shown that it consists of a replication-competent helper virus (designated REV) that is responsible for transformation. We used restriction endonuclease mapping and heteroduplex analysis to characterize the proviral DNAs of REV-A and REV. Both producer and nonproducer transformed chicken spleen cells were used as sources of REV proviral DNA; this genome was mapped in detail, and fragments of it were cloned in lambdagtWES-lambdaB. The restriction maps and heteroduplexes of the REV and REV-A genomes showed that (i) REV contains a large fraction of the REV-A gag gene; (ii) most or all of REV-A pol is deleted in REV; (iii) REV contains a 1.1 kilobase segment derived from the 3' end of REV-A pol or the 5' end of env or both; (iv) this env region in REV is followed by a 1.9-kilobase segment which is unrelated to REV-A; and (v) the helper-unrelated segment of REV extends essentially all of the way to the beginning of the 3' long terminal repeat. Therefore, REV appears to be an env gene recombinant. We also found that the REV-specific segment is derived from avian DNA, for a cloned Therefore, like the other acute transforming viruses, REV appears to be the product of recombination between a replication-component virus and host DNA. Two other defec-

tive genomes in virus-producing chicken cells were also cloned and characterized. One was very similar to REV in its presumptive gag and env segments, but instead of a host-derived insertion it contained additional env sequences. The second was similar to the first in its gag and env regions and appeared to contain an additional 1-kilobase inversion of REV-A sequences. (46 Refs)

27. GERM-LINE MULV REINTEGRATIONS IN AKR/J MICE.

Herr W, Gilbert W

Dept. Biochemistry and Molecular Biology, Biological Laboratories, Harvard Univ., Cambridge, MA, 02138

Nature; 296(5860):865-868 1982

Murine leukemia proviruses in AKR/J mice were analyzed. Enzyme digestion produced four different homozygous hybridization patterns from individual AKR/J mice. The ecotropic virus-specific hybridization probe corresponded to the glycoprotein 70 region of the ecotropic retrovirus AKV (site of integrated ecotropic proviruses). All AKR mice carried 50-, 12-, and 11-kilobase (kb) ecotropic EcoRI fragments. In addition, AKR/J mice carrying a 14-or 17-kb EcoRI fragment were observed. Patterns were noted in which either the 14-or 17-kb fragment was heterozygous. All five proviral EcoRI restriction fragments seemed to contain a full-length AKV or closely related genome. To examine cellular sequences on each side of the provirus, a combination of enzymes, one cutting inside the provirus, and a specific probe that lay on a known side of the cut, were used. Twenty-one pedigreed mice from the AKR/J pedigree expansion colony were used to examine the distribution of ecotropic proviruses. The 17-kb fragment was present in 22/42 haploid genomes examined and the 14-kb fragment was found in 19. Results indicated no linkage between loci and suggested that the 17-and 14-kb proviruses arose concurrently. The AKR/J mice seemed to be undergoing an amplification of the ecotropic proviruses. The results indicated that AKR/J mice have three common proviruses and two additional ecotropic loci scattered through the AKR population. (21 Refs)

28. CONTINUING GERM LINE INTEGRATION OF AKV PROVIRUSES DURING THE BREEDING OF AKR MICE AND DERIVATIVE RECOMBINANT INBRED STRAINS.

Steffen DL, Taylor BA, Weinberg RA

Worcester Foundation Experimental Biology, Shrewsbury, MA, 01545

J Virol; 42(1):165-175 1982

The gel electrophoresis-hybridization technique of Southern was used to analyze genetically transmitted proviruses coding for the AKV strain of murine leukemia virus. We were able to identify the restriction endonuclease EcoRI fragments containing two previously unidentified, genetically transmitted AKV proviruses of AKR mice. Comparison of different sublines of AKR mice revealed considerable heterogeneity in their complement of germ line proviruses. This heterogeneity provides evidence that the provirus complement of AKR mice is not stable. Rather, the number of genetically transmitted proviruses increases during inbreeding. Examination of a series of sublines of the C3H strain indicated that this amplification is dependent on viremia. We estimate that, in viremic strains of mice, one new provirus becomes fixed in the germ line every 15 to 30 yr. (Author abstract) (26 Refs)

29. EVIDENCE FOR THE HORIZONTAL ACQUISITION OF MURINE AKR VIROGENES BY RECENT HORIZONTAL INFECTION OF THE GERM LINE.

O'Brien SJ, Moore JL, Martin MA, Womack JE

Lab. Viral Carcinogenesis, NCI, NIH, Frederick, MD, 21701

J Exp Med; 155(4):1120-1132 1982

Estimations of the genetic distance based on 51 randomly selected biochemical loci between Mus musculus molossinus, AKR, C58, NIH Swiss, and 12 other mouse strains were performed in order to determine the origin of murine AKR virogenes. By comparing the composite allozyme genotype of various inbred and outbred mouse strains, the genetic distance statistic was derived. The genetic distance measures the degree of allelic substitution between populations and increases proportionally with the amount of time the populations have been reproductively isolated. The genetic distance determined between molossinus and all the inbred strains, including the virus-positive strains was significantly larger (3-20x) than any of the measurements between consanguinity of the strains. Restriction enzyme digestion of liver DNA from molossinus, AKR, and inbred strains that express ecotropic murine leukemia virus was used to analyze cellular DNA sequences that flank the integrated AKV provirus. Molossinus cell DNA did not contain the integration flanks of three AKR provirus sequences, while it contained at least six different proviral integration fragments. These results are easiest explained by a recent germ line infection of a progenitor of AKR by a molossinus virus, and exclude the interpretation of consanguinity of AKR and molossinus. (47 Refs)

30. LEUKEMOGENESIS BY BOVINE LEUKEMIA VIRUS: PROVIRAL DNA INTEGRATION AND LACK OF RNA EXPRESSION OF VIRAL LONG TERMINAL REPEAT AND 3' PROXIMATE CELLULAR SEQUENCES.

Kettmann R, Deschamps J, Cleuter Y, Couez D, Burny A, Marbaix G

Dept. Molecular Biology, Univ. Brussels, 67, rue des Chevaux, 1640 Rhode St-Genese, Belgium

Proc Natl Acad Sci USA; 79(8,part1):2465-2469 1982

The DNA from 17 lymphoid tumors induced by bovine leukemia virus (BLV) was digested with the restriction endonuclease EcoRI. Filter hybridization analysis using radioactive probes specific for the BLV genome showed that all tumors contained at least one or a portion of one provirus. Digestion of these proviruses with Sac I demonstrated that deletions occurred in about 25% of the cases and involved sequences located in the 5' half of the provirus. No sequence homology was observed between the cloned proximate cellular sequences flanking two different proviruses at their 3' end and the corresponding sequences in 16 other tumor DNAs, thus showing that a wide range of genomic sites could accommodate BLV proviruses. Transcription of viral DNA including long terminal repeated sequences was not detected, strongly suggesting that viral gene expression is not required for maintenance of the tumor state. No expression of 3' proximate cellular sequences was observed, indicating that no proximate downstream promotion took place in the cases examined. (Author abstract) (18 Refs)

31. CELLULAR SEQUENCES RELATED TO THREE NEW ONC GENES OF AVIAN SARCOMA VIRUS (FPS, YES, AND ROS) AND THEIR EXPRESSION IN NORMAL AND TRANSFORMED CELLS.

Shibuya M, Hanafusa H, Balduzzi PC
Rockefeller Univ., New York, NY, 10021
J Virol; 42(1):143-152 1982

Two onc genes of avian sarcoma viruses unrelated to the src gene have recently been identified: fps of Fujinami sarcoma virus/PRCII/UR1 and yes of Y73/Esh sarcoma virus. In the first part of this study we demonstrated that UR2, the most recently isolated avian sarcoma virus, contains in its genome a unique sequence, ros, nonhomologous to src, fps, and yes sequences or to transforming genes of avian acute leukemia viruses. Using cDNAs specific to the inserts of avian sarcoma virus genomes, we examined the existence and the transcription of cellular nucleotide sequences related to the three new onc genes of avian sarcoma virus (fps, yes and ros) in various cells. The progenitor cellular sequences for these onc genes (c-onc) were present in uninfected chicken DNA in one or few copies per haploid genome. These c-onc sequences were detectable in cellular DNA of a wide variety of vertebrates, and the homology between viral and cellular onc was inversely related to the phylogenetic distance of animal species. The pattern of expression of these c-onc genes in different tissues of chickens was found to be unique to each gene. The expression of c-fps and c-ros genes was generally repressed in many tissues, but c-fps was expressed at higher levels in bone marrow (2.5 copies per cell) and lung (1.1 copies per cell), whereas c-ros was mainly transcribed in kidney (2.5 copies per cell). On the other hand, c-yes transcripts were easily detectable in all tissues analyzed and were found at high levels in kidney (26 copies per cell). These c-onc expressions were unaffected by infection with avian sarcoma viruses that contained other onc genes. In a few cultures of chicken and quail transformed cells derived from tumors induced by chemical carcinogens, we found that the levels of transcription of the four c-onc genes remained unaltered, compared with that in normal tissues. (Author abstract) (60 Refs)

32. TEMPERATURE-SENSITIVE CHANGES IN THE STRUCTURE OF GLOBIN CHROMATIN IN LINES OF RED CELL PRECURSORS TRANSFORMED BY TS-AEV.

Weintraub H, Beug H, Groudine M, Graf T
Fred Hutchinson Cancer Res. Center, 1124 Columbia St., Seattle, WA, 98104
Cell; 28(4):931-940 1982

Chicken bone marrow cells infected in vitro with a temperature-sensitive avian erythroblastosis virus fail to produce hemoglobin at 36 C. When the product or products of the transforming gene (erb) are inactivated by a temperature shift to 42 C in culture, several different cloned lines of cells infected with the temperature-sensitive avian erythroblastosis virus begin to make hemoglobin. This shift in phenotype correlates with an increase in hemoglobin mRNA specific to both adult and embryonic alpha and beta globin. The switch is accompanied by the acquisition of DNAase I-hypersensitive sites in one cell line (clone 2); however, a hypothetically more mature line (clone 3) has already acquired globin DNAase-hypersensitive sites but does not express hemoglobin until the temperature shift. Several (but not all) specific restriction sites associated with both the alpha and beta domains become unmethylated after the switch from 36 C to 42 C. The magnitude of these methylation switches is small compared with changes that occur in these genes during normal avian erythropoiesis. The results suggest that changes in chromosomal structure precede transcription and are not a consequence of transcription. Since (presumptive) precursor cloned lines can be established with some, but not all, of the structural properties of active globin chromatin, it is likely that many of these properties can be independently established and are not obligatorily related. (Author abstract) (41 Refs)

ANTIVIRALS

33. MOLONEY MURINE SARCOMA VIRUS TUMORS IN CBA/J MICE: CHEMOPREVENTIVE AND CHEMOTHERAPEUTIC ACTIONS OF SUPPLEMENTAL BETA-CAROTENE.

Seifter E, Rettura G, Padawer J, Levenson SM
Dept. Biochemistry, Albert Einstein Coll. Medicine, Yeshiva Univ., Bronx, NY, 10461
JNCI; 68(5):835-840 1982

Decreased tumor frequency, increased latent period, and increased rate of tumor regression were observed in male inbred CBA/J mice fed supplemental beta-carotene before and/or after they were inoculated with the Moloney sarcoma virus. When beta-carotene feeding was begun after tumors were already present, it markedly increased the rate of tumor regression. Beta-carotene minimized the virus-induced thymus gland involution that accompanies tumor growth, and this action on the thymus gland was believed to underlie part of beta-carotene's antitumor activity. The basal diet, a standard commercial mouse chow containing more vitamin A than the National Research Council recommends as a daily allowance for rodents, supported normal growth, reproduction, and longevity of normal mice. The work reported here is the first demonstration of the antitumor action of carotene in mice inoculated with an oncogenic virus. (Author abstract) (34 Refs)

34. ABROGATION OF INFECTIVITY OF MOUSE MAMMARY TUMOR VIRUS BY RESERPINE.

Wunderlich V, Zotter S
Zentralinstitut fur Krebsforschung, Akademie der Wissenschaften der DDR, Lindenberger Weg 70/80, DDR-Berlin, E. Germany
Exp Pathol; 21(1):59-61 1982

Treatment of mouse mammary tumor virus (MMTV) with reserpine in vitro or administration of reserpine 30 min after MMTV inoculation abolishes the viral infectivity in mice. (Author abstract) (14 Refs)

35. THE ONCOSTATIC EFFECT OF METHYL-CCNU ON VARIOUS EXPERIMENTAL LYMPHORETICULAR NEOPLASMS.

Peled A, Haran-Ghera N, Chirigos MA
Dept. Animal Science, Faculty Agriculture, Hebrew Univ. Jerusalem, Rehovat, Israel
Leuk Res; 6(1):89-95 1982

Effects of semustine (methyl-CCNU) on various murine transplantable and radiation-induced leukemias were studied. The optimal dose range was 30-33 mg/kg, which could be administered with effective results from 5-18 days after tumor inoculation. Methyl-CCNU was effective against transplanted lymphatic leukemias in two strains of mice, regardless of cell markers, Thy.1 or immunoglobulin determinants and Fc recep-

tor, or genetic origin. However, against one slow- growing radiation leukemia virus (RadLV)-induced T leukemia, methyl-CCNU was almost ineffective. methyl-CCNU cured 100% of animals with myeloid leukemia and was effective (70-85%) against preleukemic cells induced by radiation or RadLV. With SJL/J mice, which have a high incidence of spontaneous reticulum cell neoplasms, methyl-CCNU was effective against transplanted leukemia and also against their spontaneous tumors. Other studies of methyl-CCNU are reviewed, all of which lead to the conclusion that methyl-CCNU has high oncostatic potential. (19 Refs)

36. DIVERSE EFFECTS: AUGMENTATION, INHIBITION, AND NON-EFFICACY OF 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA) ON RETROVIRUS GENOME EXPRESSION IN VIVO AND IN VITRO.

Lipp M, Scherer B, Lips G, Brandner G, Hunsmann G
Institut fur Virologie, Zentrum fur Hygiene, Postfach 820, D-7800 Freiburg, W. Germany
Carcinogenesis; 3(3):261-265 1982

The action of 12-O-tetradecanoylphorbol-13-acetate (TPA) on several retrovirus-related functions was investigated in four virus-host cell systems. The following effects were recorded: (i) in STU-mice, infected with the Friend virus complex (Friend murine leukemia virus/Friend spleen focus forming virus) and treated with TPA (50 ng/g) for 1 wk prior to infection, the number of spleen foci increased five-fold over the control. (ii) Addition of TPA (0.04 to 40 ng/ml) to virus-producing cell systems resulted in a two-fold increase of extracellular reverse transcriptase activity. The max response was observed in Friend leukemia virus-producing mouse cells at 0.1 to 0.4 ng TPA/ml and in simian sarcoma virus-producing rat cells at 4 ng/ml. (iii) The efficiency of transformation of BalbC 3T3 cells by Moloney murine sarcoma virus, tested in a focus formation assay, was slightly enhanced by TPA. (iv) TPA inhibited the induction of endogenous virus formation in B cell mitogen-stimulated spleen cell cultures from BalbC mice. (Author abstract) (18 Refs)

37. MURINE LEUKEMIA VIRUS-MEDIATED TRANSFORMATION OF A MOUSE EPITHELIAL CELL LINE MMC-E.

Rapp UR, Keski-Oja J
Lab. Viral Carcinogenesis, NCI, Building 560, Room 21-77, Frederick, MD, 21701
Cancer Res; 42(6):2407-2411 1982

Mouse embryo epithelial cells, MMC-E, were malignantly transformed in culture with MuLV derived from C3H/MCA 5 cells chemically induced by 5-iododeoxyuridine. The frequency of transformation was significantly enhanced if infection was followed by treatment with 100 ng of 12-O-tetradecanoylphorbol-13-acetate per ml. The virus-transformed cells retained their high plating efficiency in soft agar in the absence of 12-O-tetradecanoylphorbol-13-acetate and formed neoplasms in nude mice. (Author abstract) (24 Refs)

38. EFFECT OF MOUSE INTERFERON ON CELL TRANSFORMATION AND VIRUS PRODUCTION IN RAT CELLS EXOGENOUSLY INFECTED WITH MOLONEY MURINE SARCOMA AND LEUKEMIA VIRUSES.

Huleihel M, Aboud M
Microbiology and Immunology Unit, Faculty Health Sciences, Ben Gurion Univ. Negev, Beer Sheva, Israel
Int J Cancer; 29(4):471-476 1982

Foci of transformed cells, produced by MSV(124), appeared to result only from the primary infection, since this virus stock yielded a virus-nonproducing infection. On the other hand, the majority of foci scored in MSV/MLV-infected cultures, were generated by multiple secondary infections with the progenies of the primary infection. Mouse interferon (IF) was highly inhibitory for cell transformation by both virus stocks. However, this inhibition was apparent in MSV(124) infected cultures only if IF was added at least 12 hr before infection, whereas in MSV/MLV-infected cultures IF was highly effective even if added 24 hr after infection. The inhibition of focus formation by MSV(124) was irreversible after removal of IF, suggesting that IF inhibited an early step before provirus integration into the host genome. By contrast, in MSV/MLV-infected cultures focus formation was almost completely restored after recovery from the IF effect. Nevertheless, examination of virus production after IF removal proved that in MSV/MLV infection, too IF exerted an inhibitory effect before provirus integration. (Author abstract) (38 Refs)

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VIRAL PROTEINS

1. PRECURSOR POLYPEPTIDES TO STRUCTURAL PROTEINS OF VISNA VIRUS.

Vigne R, Filippi P, Querat G, Sauze N, Vitu C, Russo P, Delori P
Laboratoire de Virologie, Faculte de Medecine Nord, 13326 Marseille, Cedex 3, France
J Virol; 42(3):1046-1056 1982

Visna virus is a retrovirus which replicates in fibroblast-like cells of the sheep choroid plexus through a lytic cycle. Visna virions contain three major low-mol-wt proteins (p30, p16, and p14) which partially constitute the core structure of the virions. By analogy with the oncoviruses, the internal proteins, envelope glycoprotein (gp135), and reverse transcriptase are probably encoded by the gag, env, and pol genes respectively. To elucidate the genetic organization of the visna virus genome and its expression, we studied the synthesis of viral proteins in infected sheep choroid plexus cells. Intracellular viral proteins were detected by immunoprecipitation of pulse-labeled cell extracts with monospecific sera raised against p30, p16, and gp135 and resolution of the proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunoprecipitation with anti-p30 and anti-p16 sera allowed the characterization of the 55,000-dalton polypeptide precursor to internal virion proteins p30, p16, and p14 (Pr55gag). Tryptic peptide mapping confirmed the precursor-product relationship between Pr55gag and the three internal proteins. In addition, a gag-related polypeptide of 150,000 daltons was also detected. This polypeptide, which was less abundant than Pr55gag is a likely precursor to the viral reverse transcriptase (Pr150gag-pol). Pr55gag and Pr150gag-pol are not glycosylated. The precursor related to major envelope protein gp135 is a glycosylated polypeptide with an av mol wt of 150,000 (gPr150env). Pulse-chase experiments indicated that gPr150env matures into glycoprotein gp135 intracellularly; however, gp135 was never

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preponderant in cell extracts. The non-glycosylated form of gPr150env, which accumulated in the presence of 2-deoxy-D-glucose, appeared as a polypeptide of about 100,000 daltons. These results indicated that visna virus codes for the largest non-glycosylated env-related precursor among all of the retroviruses and therefore probably contains the largest env gene. (30 Refs)

2. VIRAL PROTEINS AND SITE-SPECIFIC CLEAVAGE.

Korant BD, Lonberg-Holm KK
Central Res. and Development Dept., Du Pont Experimental Station, Wilmington, DE, 19898
Acta Biol Med Ger; 40(10/11):1481-1488 1981

Recent developments in the intracellular site-specific cleavage of polyviral proteins are summarized and the results indicating that the host can regulate these processing reactions are described. The reported properties for two animal proteases are listed; those of picornaviruses and RNA tumor viruses, and are compared to the protease of T4, a DNA bacteriophage. The presumed function of the virus-coded proteases is to carry out cleavages which the host cell cannot accomplish. Using mainly empirical approaches, a variety of non-specific ways to block viral protein processing have been identified. In situ protease assay for virus mutants is presented. The rat ribosomal protease when incubated with viral cytoplasmic proteins, readily degrades them, as it also degrades amino acid analog-containing cellular proteins. Host regulators of viral proteolysis are macromolecular protein-protease inhibitors from the serum of healthy animals. An inhibitor in human serum which reacts with poliovirus protease is alpha2 macroglubulin, however rat alpha2 macroglubulin is the best inhibitor detected to date. The complexing ability of the alpha2M antiprotease is being used to prepare quantities of polioviral protease sufficient for primary sequence analysis confirming the genetic locus of the protease on the viral RNA genome. (14 Refs)

3. ML ANTIGEN OF DBA/2 MOUSE LEUKEMIAS: EXPRESSION OF AN ENDOGENOUS MURINE MAMMARY TUMOR VIRUS.

Racevskis J, Sarkar NH
Lab. Molecular Virology, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
J Virol; 42(3):804-813 1982

Spontaneous, transplantable leukemias of DBA/2 mice express an antigen (ML) which cross-reacts with antigens of murine mammary tumor virus (MuMTV). The MuMTV cross-reactive antigen of the DBA/2 leukemias (ML cells) was found to be a glycoprotein of 78,000 mol wt containing antigenic determinants of the major MuMTV glycoprotein gp52. No MuMTV particles were produced by the ML cells, although they did contain type A particles - the pronucleocapsids of MuMTV. The ML antigen appeared to be an aberrant form of the intracellular MuMTV env precursor molecule prgp70, which was not processed properly but instead acquired extra carbohydrate groups and was expressed in uncleaved form on the cell surface. Isolation of MuMTV core protein p28 from the leukemic cells and subsequent tryptic peptide mapping analysis showed that the p28 from leukemia cells differed from the p28 of MuMTV isolated from DBA/2 mouse milk. These observations indicate that the MuMTV expressed in DBA/2 leukemic spleen cells is of a different strain than the virus secreted in lactating mammary glands of DBA/2 mice and probably represents the expression of an endogenous DBA/2 provirus. (Author abstract) (34 Refs)

4. ROLE OF P60SRC KINASE ACTIVITY IN THE INDUCTION OF NEURORETINAL CELL PROLIFERATION BY ROUS SARCOMA VIRUS.

Poirier F, Calothy G, Karess RE, Erikson E, Hanafusa H
Institut Curie, Centre Universitaire, 91405-Orsay, France
J Virol; 42(3):780-789 1982

Expression of the src gene of Rous sarcoma virus (RSV) in chicken embryo neuroretinal (NR) cells results in morphological transformation and sustained proliferation of a normally resting cell population. We have previously reported the isolation of mutants of RSV which retain full growth-promoting activity while displaying reduced transforming properties. Two such mutants, PA101 and PA104, were used to investigate whether the p60src-associated kinase activity is required for the mitogenic function of src. A comparison of the patterns of phosphorylation of wild-type and mutant p60src revealed that the phosphorylation of tyrosine residues of p60src of PA104 was markedly reduced, whereas the relative amount of phosphotyrosine in p60src of PA101 was comparable to that of the wild-type protein. In vitro kinase activity of p60src immunoprecipitated from NR cells infected with PA101 or PA104 as measured by phosphorylation of the heavy chains of specific immunoglobulin G molecules was 1/10 that of the wild-type molecule. Moreover, when NR cells infected with mutants temperature sensitive for mitogenic capacity were maintained at a temperature either permissive or restrictive for cell growth, quantitation of kinase activity indicated that proliferation of NR cells could not be linked to the absolute level of in vitro kinase activity of p60src. Transformation of NR cells by wild-type RSV resulted in a 10-fold increase in total cellular phosphotyrosine and in the phosphorylation of tyrosine residues of a 34K protein, a possible in vivo substrate for p60src. In contrast, phosphorylation of tyrosine residues of cellular targets was markedly reduced in NR cells infected with PA101 or PA104. These results indicate that the mitogenic capacity of RSV in NR cells does not require elevated levels of p60src kinase activity. (Author abstract) (27 Refs)

5. VIRAL SRC GENE PRODUCTS ARE RELATED TO THE CATALYTIC CHAIN OF MAMMALIAN CAMP-DEPENDENT PROTEIN KINASE.

Barker WC, Dayhoff MO
Natl. Biomedical Res. Foundation, Georgetown Univ. Medical Center, Washington, DC, 20007
Proc Natl Acad Sci USA; 79(9):2836-2839 1982

The transforming protein sequences translated from the Rous avian and Moloney murine sarcoma virus src genes are shown to be related to the catalytic chain of bovine cAMP-dependent protein kinase (ATP: protein phosphotransferase, EC 2.7.1.37). The avian transforming protein, also a protein kinase, shows greatest homology with the bovine protein kinase in the carboxyl-terminal half, where the protein kinase activity is localized. Moreover, lysine occurs in the inferred transforming protein sequences at the position homologous with the proposed ATP-binding lysine of the bovine protein kinase. This relationship is consistent with the hypothesis that the src genes originated in the host genomes, in which they are members of a superfamily of distantly related protein kinases that are normal constituents of mammalian cells. In the host, these sequences are much more highly conserved than in the viruses. (Author abstract) (16 Refs)

VIRAL RNA

6. ACCUMULATION OF SPLICED AVIAN RETROVIRUS MRNA IS INHIBITED IN S-ADENOSYLMETHIONINE-DEPLETED CHICKEN EMBRYO FIBROBLASTS.

Stoltzfus CM, Dane RW
Dept. Microbiology, Univ. Iowa, Iowa City, IA, 52242
J Virol; 42(3):918-931 1982

The synthesis and processing of B77 avian sarcoma virus RNA in infected chicken embryo fibroblasts was followed in the presence and absence of cycloleucine, a competitive inhibitor of the synthesis of S-adenosylmethionine and thus an inhibitor of RNA methylations. An increase in the steady-state levels of genome-length RNA and a decrease in the steady-state levels of subgenomic RNA molecules were obtained in the S-adenosylmethionine-depleted avian sarcoma virus-infected cells. The total number of virus-specific RNA molecules per cell, however, remained relatively constant under either condition. The production of newly synthesized virus-specific RNA in cycloleucine-treated and untreated cells infected with a transformation-defective strain of B77 avian sarcoma virus was followed as a function of [3H]uridine labeling time. The accumulation of radioactive genome-length 8.4-kilobase (kb) RNA continued in cycloleucine-treated cells, and virus particle production proceeded at normal rates. Newly synthesized 3.5-kb subgenomic mRNA, the putative mRNA for the envelope protein precursor, failed to accumulate in the treated cells. The extent of the inhibition in the appearance of the radioactive 3.5-kb RNA was correlated with the extent of the inhibition of viral genomic and cellular mRNA methylations and was a function of the cycloleucine concentration. When the accumulation of 3.5-kb envelope protein mRNA was blocked by the cycloleucine treatment, there were significant increases in the rate of synthesis of the polypeptide products of the genome-length RNA, the precursors to the non-glycosylated gag proteins and the reverse transcriptase relative to the rate of synthesis of the envelope protein precursor. These results suggest that there is an S-adenosylmethionine requirement for the splicing, but not for the synthesis, packaging, or messenger function, of avian retrovirus genome-length RNA. Possible reasons for this requirement are discussed. (47 Refs)

7. METABOLISM OF FAST LABELING NUCLEAR RNA IN SPLEEN CELLS OF MICE WITH RAUSCHER LEUKEMIA.

Smirnova IA, Kishinskaia EG
R. E. Kavetskii Inst. Problem Oncology, Kiev, USSR
Ukr Biokhim Zh; 54(2):159-162 1982

Synthesis and degradation of fast labeling RNA with sedimentation coefficient of 35 Svedberg (S) were studied in splenocytes from BALB/c mice infected with Rauscher leukemia virus (RLV). The 35 S RNA was characterized by a higher metabolic activity than other RNA fractions in leukemic cells. Molecular hybridization showed that 35 S RNA consisted of the sequences which were complementary to RLV genome and to the cell genome. (10 Refs) (In RUS)

8. NUCLEOTIDE SEQUENCE OF 7 S RNA. HOMOLOGY TO ALU DNA AND LA 4.5 S RNA.

Li WY, Reddy R, Henning D, Epstein P, Busch H
Dept. Pharmacology, Baylor Coll. Medicine, Houston, TX, 77030
J Biol Chem; 257(5):5136-5142 1982

The 7 S RNA component, a part of normal higher eukaryotic cells and several oncornaviruses, was shown to be conserved in evolution. Recently, 7 S RNA was shown to be partially complementary to Alu family DNA sequences. In the present study the nucleotide sequence of Novikoff hepatoma 7 S RNA was determined. Comparison of 7 S RNA, Alu and B1 family DNA, and La 4.5 S RNA sequences for homologies showed that (1) one-third of 7 S RNA, mainly the 5'-end, was homologous to Alu and B1 family sequences; (2) one 300-nucleotide long Alu family sequence contained two binding sites for 7 S RNA; and (3) the 5'-ends of 7 S RNA and La 4.5 S RNA also had extensive (60%) homologies. A model for the secondary structure of 7 S RNA based on maximal base pairing and preferential nuclease cleavage sites is also presented. (33 Refs)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

9. AVIAN LYMPHOID LEUKOSIS VIRUS INFECTION AND DNA INTEGRATION IN THE PRELEUKOTIC BURSAL TISSUES: A COMPARATIVE STUDY OF SUSCEPTIBLE AND RESISTANT LINES.

Fung YT, Fadly AM, Crittenden LB, Kung HJ
(c/o Kung) Dept. Biochemistry, Michigan State Univ., East Lansing, MI, 48824
Virology; 119(2):411-421 1982

Lymphoid leukosis (LL) is a naturally occurring neoplastic disease of chickens, caused by avian lymphoid leukosis virus (ALV). Most inbred lines of chickens, susceptible to virus infection, are also susceptible to LL development. An interesting exception is line 6(3) which rarely develops LL after virus infection. To obtain more information concerning the resistance of line 6(3) to LL, we have compared virus infection and integration in bursal tissues of resistant line 6(3) and a susceptible cross (15I(5) x 7(1)) after virus inoculation into day-old chicks. We report here that (a) the bursa tissues in both lines are infected to similar extent and (b) the initial viral infections in both lines lead to 'random' integration of proviruses in the chromosomes of the bursal tissues. Only in the tumors developing later in the susceptible line can specific integration of provirus near a cellular oncogene, c-myc, be detected. Based on these data, the mechanism of resistance of line 6(3) to LL is discussed. (Author abstract) (26 Refs)

10. SUSCEPTIBILITY TO PRODUCTIVE INFECTION BY THE BOVINE LEUKEMIA VIRUS IS GOVERNED AT THE PROVIRAL LEVEL (MEETING ABSTRACT)

Casey JW, Nicolson MO
Louisiana State University Medical Center, New Orleans, LA
Fed Proc; 41(3):1275 1982

The three BLV proviruses present in a productively infected monoclonal sheep cell line (FLK-BLV) have been cloned into the modified vector L47. These isolates are 8.7KB in length and have identical restriction endonuclease sites. Hybridization patterns obtained from SmaI, XhoI, HpaII and MspI digested FLK-BLV DNA indicate that 2 of the 3 BLV proviruses are extensively modified. Five cell lines, human RD-4, BAT-B88, Dog D17, beagle and guinea pig were infected with BLV at a MOI of approximately 3. In each case no productive infection was observed as monitored by reverse transcriptase activity. However, DNA extracted from these abortively infected cell lines contain an average of 1-2 BLV proviruses per cell. Con-

sidering the difficulty we and others have found in establishing productive infection in a wide range of cell lines and the observation that BLV is not expressed in the B lymphocytes of infected cattle, we propose that BLV integrates into domains of host sequences that normally repress its expression. (no Refs)

11. MOLECULAR CLONING OF THE FUJINAMI SARCOMA VIRUS GENOME AND ITS COMPARISON WITH SEQUENCES OF OTHER RELATED TRANSFORMING VIRUSES.

Shibuya M, Wang LH, Hanafusa H
Rockefeller Univ., New York, NY, 10021
J Virol; 42(3):1007-1016 1982

Full-length proviral DNA of Fujinami sarcoma virus (FSV) of chickens was molecularly cloned and characterized. An analysis of FSV DNA integrated in mammalian cells showed that restriction endonuclease SacI has a single cleavage site on FSV DNA. Unintegrated closed circular FSV DNA obtained from newly infected cells was linearized by digestion with SacI and cloned into lambdagtWES-lambdaB. The following three different molecules were isolated: FSV-1 (4.4 kilobases [kb]) and FSV-2 (4.7 kb), which appeared to be full-length FSV DNA molecules containing either one or two copies of the long terminal repeat structure, and FSV-3 (6 kb), which consisted of part FSV DNA and part DNA of unknown origin. An analysis of the structure of cloned FSV-1 and FSV-2 DNA molecules by restriction endonuclease mapping and hybridization with appropriate probes showed that about 2.6 kb of the FSV-unique sequence called FSV-fps is located in the middle of the FSV genome and is flanked by helper virus-derived sequences of about 1.3 kb at the 5' end and 0.5 kb at the 3' end. The long terminal repeats of FSV were found to have no cleavage site for either EcoRI or PvuI. Upon transfection, both FSV-1 DNA and FSV-2 DNA were able to transform mammalian fibroblasts. Four 32P-labeled DNA fragments derived from different portions of the FSV-fps sequence were used for hybridization to viral RNAs. We found that sequences within the 3' half of the FSV-fps gene are homologous to RNAs of PRCII avian sarcoma virus and the Snyder-Theilen strain of feline sarcoma virus, both of which were previously shown to contain transforming genes related to FSV-fps. These results suggest that the 3' portion of the FSV-fps sequence may be crucial for the transforming activity of fps-related oncogenic sequences. (Author abstract) (42 Refs)

12. ISOLATION AND CHARACTERIZATION OF C-MYC, A CELLULAR HOMOLOG OF THE ONCOGENE (V-MYC) OF AVIAN MYELOCYTOMATOSIS VIRUS STRAIN 29.

Vennstrom B, Sheiness D, Zabielski J, Bishop JM
Dept. Microbiology, Univ. Uppsala, Biomedical Center, Uppsala, Sweden
J Virol; 42(3):773-779 1982

The chicken genome contains nucleotide sequences homologous to the transforming genes (oncogenes) of a number of avian retroviruses. We have isolated chicken DNA (c-myc) that is homologous to the oncogene (v-myc) of the avian myelocytomatosis virus MC29 and have compared the structures of the cellular and viral genes. Results from restriction endonuclease mapping of c-myc and from analysis of heteroduplexes between the DNAs of the cellular and viral genes show that c-myc is homologous to 1,500 nucleotides in v-myc DNA. This homologous region is interrupted in c-myc by an intron-like sequence of 1,100 nucleotides which is absent from v-myc. Nuclear RNA from normal chicken cells contains at least five species of transcripts from c-myc ranging from 2.5 to 6.5 kilobases in length. By contrast, cytoplasm contains only the 2.5-kilobase c-myc RNA. These features of the c-myc gene and its nuclear transcripts are characteristic of normal cellular genes and suggest that the myc gene is of cellular rather than viral origin. The exons in c-myc may define two functional domains in the gene and may therefore facilitate the dissection of the different oncogenic potentials of the MC29 virus. (Author abstract) (28 Refs)

13. ISOLATION OF HUMAN ONCOGENE SEQUENCES (V-FES HOMOLOG) FROM A COSMID LIBRARY.

Groffen J, Heisterkamp N, Grosveld F, Van de Ven W, Stephenson JR
Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
Science; 216(4550):1136-1138 1982

To define the human homolog (or homologs) of transforming sequences (v-fes gene) common to Gardner (GA) and Snyder Theilen (ST) isolates of feline sarcoma virus (FeSV), a representative library of human lung carcinoma DNA in a cosmid vector system was constructed. Three cosmid clones were isolated containing GA/ST FeSV v-fes homologous cellular sequences, within 32-to 42-kilobase cellular inserts representing 56 kilobases of contiguous human cellular DNA. Sequences both homologous to, and colinear with, GA or ST FeSV v-fes are distributed discontinuously over a region of up to 9.5 kilobases and contain a minimum of three regions of nonhomology representing probable introns. A thymidine kinase selection system was used to show that, upon transfection to RAT-2 cells, the human c-fes sequence lacked detectable transforming activity. (Author abstract) (24 Refs)

14. INFECTIVITY AND STRUCTURE OF MOLECULAR CLONES OBTAINED FROM TWO GENETICALLY TRANSMITTED MOLONEY LEUKEMIA PROVIRAL GENOMES.

Harbers K, Schnieke A, Stuhlmann H, Jaenisch R
Heinrich-Pette-Institut fur Experimentelle Virology und Immunology, Universitat Hamburg, Martinistrasse 52, 2000 Hamburg 10, W. Germany
Nucleic Acids Res; 10(8):2521-2537 1982

The Mov-2 and Mov-10 substrains of mice, each carrying Moloney leukemia virus (= M-MuLV) in their germ line at the Mov-2 and Mov-10 locus, respectively, do occasionally at a later age (Mov-2) or not at all (Mov-10) activate infectious virus. The M-MuLV proviruses with flanking mouse sequences corresponding to the Mov-2 and Mov-10 locus, respectively, were molecularly cloned. Restriction enzyme analysis revealed no major deletions or insertions in the proviral genomes of the Mov-2 and Mov-10 locus. Both cloned DNAs induced XC plaques in a transfection assay. The specific infectivity, however, was very low and 3T3 cells transfected with the Mov-2 or Mov-10 clone did not produce infectious virus. Removing part of the 5' cellular sequences from the Mov-10 clone did not increase the infectivity. The results suggest that the M-MuLV integrated at the Mov-2 and Mov-10 locus carry a mutation which prevents synthesis of infectious virus but permits XC plaque induction by partial genome expression or synthesis of non-infectious particles. (Author abstract) (28 Refs)

15. CLONING OF TWO GENETICALLY TRANSMITTED MOLONEY LEUKEMIA PROVIRAL GENOMES: CORRELATION BETWEEN BIOLOGICAL ACTIVITY OF THE CLONED DNA AND VIRAL GENOME ACTIVATION IN THE ANIMAL.

Chumakov I, Stuhlmann H, Harbers K, Jaenisch R
(c/o Jaenisch) Heinrich-Pette-Institut fur Experimentelle Virologie, Universitat Hamburg, 2000 Hamburg 20, W. Germany
J Virol; 42(3):1088-1098 1982

The Mov-7 and Mov-9 substrains of mice, carrying Moloney murine leukemia virus (M-MuLV) in their germ line at the Mov-7 locus and Mov-9 locus, respectively, are different with respect to virus activation. Infectious virus appears in all mice carrying the Mov-9 locus but is not activated in animals carrying the Mov-7 locus. Consequently, only Mov-9 mice develop viremia and subsequent leukemia. The endogenous M-MuLV provirus with flanking mouse sequences corresponding to the Mov-7 and Mov-9 loci was molecularly cloned. Detailed restriction maps obtained from the cloned DNAs revealed no detectable differences in the proviral genomes. The flanking mouse sequences, however, were different, confirming that the Mov-7 and Mov-9 loci represent different integration sites of M-MuLV. Both clones induced XC plaques in a transfection assay. The specific infectivity of the clones, however, was different. Moreover, NIH 3T3 cells transfected with the Mov-9 clone produced NB-tropic M-MuLV, whereas cells transfected with the Mov-7 clone did not produce infectious virus. The results suggest that M-MuLV integrated at the Mov-7 locus carries a mutation which prevents synthesis of infectious virus but permits XC plaque induction by partial genome expression or synthesis of noninfectious particles. Thus, the pattern of virus expression in Mov-7 and Mov-9 mice correlates with the biological properties of the respective clones. Genomic DNA from Mov-9 mice was not infectious in the transfection assay. As the only difference between the genomic and the cloned Mov-9 DNA appears to be the presence of 5-methylcytosine in CpG sequences, our results suggest that removal of methyl groups by molecular cloning in procaryotes permits genome expression in transfected eucaryotic cells. Our results support the hypothesis that DNA methylation is relevant not only in genome expression in the animal but also in expression of genes transfected into eukaryotic cells. (28 Refs)

16. ORIGIN, STRUCTURE, AND FUNCTION OF THE TRANSFORMING REGION ON THE ABELSON MURINE LEUKEMIA VIRUS (MEETING ABSTRACT)

Baltimore D, Wang J, Goff S, Queen C
Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA
Thirty-fifth Annual Symposium on Fundamental Cancer Research, March 2-5, 1982, Houston, Texas. The University of Texas M. D. Anderson Hospital and Tumor Institute 38 pp., 1982.

The transforming region, V-abl, of the Abelson murine leukemia virus (A-MuLV) originates in a normal gene of the mouse, C-abl. C-abl represents a number of small regions of DNA that are homologous to V-abl separated by long stretches of DNA that are not. Our interpretation of this organization is that V-abl represents a cDNA copy of the exons that compose the C-abl gene and that the large nonhomologous regions represent introns that have been excised. V-abl joins the MuLV sequence in the A-MuLV genome at a precise point where there is a four-base homology between the Moloney murine leukemia virus (M-MuLV) sequence and the C-abl sequence. The joining position appears to be in the center of an exon of the C-abl gene. At the 3' end, the V-abl/M-MuLV joint is again precise, but the C-abl sequence has not been completed in that region. The organization of the transcripts of the C-abl gene indicates that the C-abl mRNA (of which there are two) has 400 bases 5' of the region homologous to V-abl and 2 to 3 kb 3' of the homologous region. All these structural facts can be reconciled by assuming that the A-MuLV genome arose through the integration of a M-MuLV genome upstream from the C-abl gene, followed by a deletion that brought the M-MuLV sequences in apposition to a C-abl exon. Transcription of this deleted gene from the M-MuLV promoter would then generate an RNA transcript with an M-MuLV 5' end. The final A-MuLV genome presumably arose by a secondary recombination at the reverse transcription level, according to the model of Goldfarb and Weinberg. In the context of the A-MuLV genome, the V-abl sequences encode a tyrosine-specific protein kinase activity. By expressing the V-abl region in Escherichia coli, the kinase activity has been shown to be intrinsic to the V-abl protein because in Escherichia coli the protein is phosphorylated on a tryosine residue. (1 Ref)

17. GENE REGULATION AND ONCOGENESIS BY RETROVIRUSES (MEETING ABSTRACT)

Varmus HE
Department of Microbiology and Immunology, University of California, San Francisco, CA
Thirty-fifth Annual Symposium on Fundamental Cancer Research, March 2-5, 1982, Houston, Texas. The University of Texas M. D. Anderson Hospital and Tumor Institute 38 pp., 1982.

We studied the functional capacities of long terminal repeats (LTRs) by constructing recombinant plasmids, which contain LTRs and cloned genes in various arrangements, and then introducing them into cultured cells. As expected, only an LTR in the correct transcriptional orientation upstream from a promoter-less gene can produce expresson of that gene. However, when the gene carries its own promoter, the efficiency of DNA transformation is enhanced manyfold by the presence, in either orientation, of certain LTRs suggesting that some LTRs may enhance gene expression at distant, heterologous promoters in a nonpolar fashion. The LTR of mouse mammary tumor virus (MMTV) governs expression of viral or linked cellular genes in a glucocorticoid-responsive manner; the region responsible for hormonal regulation has been localized to about 0.4 kb near the 3' end of the MMTV LTR. The induction of B-cell lymphomas by avian leukosis virus (ALV) appears to require insertion of proviral DNA near c-myc, a putative cellular oncogene. The viral inserts, sometimes comprising little more than a single LTR, can enhance expression of c-myc in at least three arrangments: When positioned upstream from c-myc in the same transcriptional orientation, the inserts are promoters for the transcription of c-myc; when positioned upstream in the opposite orientation to c-myc or downstream in the same orientation, they enhance expression from unidentified cellular promoter(s). In the last instance, the LTR provides a novel site for polyadenylation. Interrupted c-myc loci have been cloned from tumor DNA and reintroduced into cultured cells to identify determinants of the enhancement phenomena. (no Refs)

ANTIVIRALS

18. DIFFERENTIAL INHIBITION OF DNA POLYMERASE AND RNASE H ACTIVITIES OF THE REVERSE TRANSCRIPTASE BY PHOSPHONOFORMATE.

Margalith M, Falk H, Panet A
Dept. Virology, Hebrew Univ., Hadassah Medical Sch., Jerusalem, Israel
Mol Cell Biochem; 43(2):97-103 1982

Three potential inhibitors of reverse transcriptase activities, phosphonoformate (PF), phosphonoacetate (PAA), and ethyldiethyl phosphonoformate (Et-PF), were compared in this study. Only PF was found to inhibit the DNA polymerase activity of the purified reverse transcriptase of Moloney murine leukemia virus (M-MuLV) and avian myeloblastosis virus (AMV). The degree of DNA polymerase inhibition was linear with PF concentration; 50% inhibition was achieved at 10 uM. Whereas PF inhibited both the RNA and DNA dependent DNA polymerase activities, the RNase H activity of the reverse transcriptase was unaffected. Both the endogenous DNA polymerase activity in detergent disrupted virus and the activity of the purified enzyme with the isolated virus genome 70S RNA were inhibited by PF. However, higher concentrations of PF were needed to inhibit the endogenous reaction. The inhibition by PF appeared to be reversible and noncompetitive with respect to the substrate deoxythymidine triphosphate (dTTP). Addition of PF after the initiation of DNA synthesis immediately arrested the reaction. (Author abstract) (26 Refs)

19. 5-MERCAPTOPOLYURIDYLIC ACID (MPU), A POTENT INHIBITOR OF THE REVERSE TRANSCRIPTASE FROM AVIAN MYELOBLASTOSIS VIRUS.

Kung MP, Ho YK, Bardos TJ
Dept. Biochemical Pharmacology, Biophysical Sciences, State Univ. New York at Buffalo, Buffalo, NY, 14260
Res Commun Chem Pathol Pharmacol; 36(2):215-228 1982

The effects of partially thiolated polyuridylic acid (MPU), partially thiolated polycytidylic acid (MPC), and their unmodified counterparts, poly(U) and poly(C), respectively, on the reverse transcriptase of avian myeloblastosis virus (AMV) have been determined, using different template-primers. Poly(C) stimulated and MPC inhibited the polymerization reaction catalyzed by the AMV reverse transcriptase in the presence of the natural viral template (endogenous RNA or purified 70S RNA), or with poly(C)-oligo(dG)12-18 but not with poly(A)-oligo(dT)12-18 as the only template-primer present in the purified enzyme system. Both poly(U) and MPU inhibited the reaction in the presence of either of the synthetic or natural templates. Using the purified enzyme with the 70S RNA template, MPU was by far the most potent inhibitor, with I50 = 0.8 uM based on the epsilon(P) value, or I50 less than 3 x 10(-9) M based on the actual, macromolecular weight of the polynucleotide. (Author abstract) (29 Refs)

20. EFFECTS OF MONENSIN ON MORPHOGENESIS AND INFECTIVITY OF FRIEND MURINE LEUKEMIA VIRUS.

Srinivas RV, Melsen LR, Compans RW
(e/o Compans) Dept. Microbiology, Univ Alabama in Birmingham, Birmingham, AL, 35294
J Virol; 42(3):1067-1075 1982

The transport of the gp70 glycoprotein to the cell surface and concomitant release of infectious virus was inhibited by treatment of Friend murine leukemia virus-infected Eveline cells with the sodium ionophore monensin. Virus yields were reduced more than 50-fold by 10(-5) M monensin, whereas particle production was reduced by 50% in monensin-treated cells. The resulting particles failed to incorporate newly synthesized gp70 and p15(E), whereas the other structural proteins, p30, p15, p12, and p10, were incorporated into virions. However, monensin did not inhibit the incorporation into virions of preformed gp70. A reduction in the efficiency of cleavage of the PrENV glycoprotein precursor and a defect in the processing of simple endo-H-sensitive to complex endo-H-resistant oligosaccharides suggest that intracellular transport of gp70 may be blocked before its entry into the Golgi apparatus. Fever particles were found to bud from the cell surface, but intracellular vacuoles with budding virions were detected. Ferritin labeling and pulse-chase studies suggested a cell surface origin for these vacuoles. These experiments indicate that monensin inhibits the transport of Friend murine leukemia virus glycoproteins at an early stage, with a resultant block in the assembly and release of infectious virus. (Author abstract) (37 Refs)

OTHER RELATED STUDIES

21. AVIAN SARCOMA VIRUS Y73 GENOME SEQUENCE AND STRUCTURAL SIMILARITY OF ITS TRANSFORMING GENE PRODUCT TO THAT OF ROUS SARCOMA VIRUS.

Kitamura N, Kitamura A, Toyoshima K, Hirayama Y, Yoshida M
(c/o Yoshida), Cancer Inst., Toshima-ku, Tokyo 170, Japan
Nature; 297(5863):205-208 1982

From the complete nucleotide sequence of the genome of the avian sarcoma virus Y73, we have predicted amino acid sequence of p90gag-yes, the product of the transforming gene. Contrary to previous evidence from molecular hybridization studies, p90gag-yes was found to have much homology with the transforming gene product p60src of Rous sarcoma virus, suggesting that the cellular counterparts of the two (c-yes and c-src) originated from a common prototype sequence. (Author abstract) (32 Refs)

22. DEMONSTRATION OF BIOLOGICAL ACTIVITY AND NUCLEOTIDE SEQUENCE OF AN IN VITRO SYNTHESIZED CLONE OF THE MOLONEY MURINE SARCOMA VIRUS MOS GENE.

Donoghue DJ
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
J Virol; 42(2):538-546 1982

A clone of the Moloney murine sarcoma virus mos gene derived by in vitro reverse transcription was characterized. When assayed for focus formation by DNA transfection on NIH/3T3 cells, this clone was biologically inactive, presumably due to the absence of a long terminal repeat sequence. Therefore, a long terminal repeat was inserted into the clone by in vitro recombination, after which the mos gene was able to transform NIH/3T3 cells efficiently. The nucleotide sequence encompassing the transforming region of this clone was determined. A single long open reading frame was observed, which potentially encodes a polypeptide of 41,000 daltons. This open reading frame initiates with the first five amino acids of the murine leukemia virus env gene, after which it enters the mos sequence, where it terminates. The nucleotide se-

quences described in this paper was compared with other sequences of mos in an effort to resolve discrepancies in the position of the long open reading frame. Although Moloney murine sarcoma virus retains the 3' splicing site of the murine leukemia virus env gene, a mos-specific mRNA which corresponds structurally to the murine leukemia virus env mRNA was not identified. The sequence described here revealed a single nucleotide change in the proposed env gene 3' splicing site which was retained in Moloney murine sarcoma virus. This deviation from the consensus 3' splicing sequence may underlie the absence of mos expression via the env gene splicing pathway. (Author abstract) (44 Refs)

23. A GENERALIZED METHOD OF SUBCLONING DNA FRAGMENTS BY RESTRICTION SITE RECONSTRUCTION: APPLICATION TO SEQUENCING THE AMINO-TERMINAL CODING REGION OF THE TRANSFORMING GENE OF GAZDAR MURINE SARCOMA VIRUS.

Donoghue DJ, Hunter T
Tumor Virology Lab., Salk Inst., P.O. Box 85800, San Diego, CA, 92138
Nucleic Acids Res; 10(8):2549-2564 1982

The technique of restriction site reconstruction was generalized so as to allow the subcloning of any DNA fragment and its subsequent reexcision with EcoRI, XbaI, XhoI or HindIII. After excision, the 3' terminus of each strand will be derived from the starting nucleic acid, permitting the use of such fragments as primers for nucleotide sequencing by primer extension methods. The technique was used to subclone a 56 base pair BstNI-DdeI fragment of Moloney murine sarcoma virus (Mo-MSV) as a unique HindIII-HindIII fragment. This fragment then served as a primer to sequence a portion of the RNA genome of Gazdar murine sarcoma virus (Gz-MSV). The nucleotide sequence which was obtained indicated that the transforming gene of Gz-MSV arose by at least two recombination events involving murine leukemia virus (MLV) and the cellular homologue c-mos. This analysis suggests that a virus indistinguishable from Mo-MSV was an intermediate in the formation of Gz-MSV. (Author abstract) (31 Refs)

24. NUCLEOTIDE SEQUENCE OF THE AKV ENV GENE.

Lenz J, Crowther R, Straceski A, Haseltine W
(c/o Haseltine), Sidney Farber Cancer Inst., Boston, MA, 02115
J Virol; 42(2):519-529 1982

The sequence of 2,191 nucleotides encoding the env gene of murine retrovirus Akv was determined by using a molecular clone of the Akv provirus. Deduction of the encoded amino acid sequence showed that a single open reading frame encodes a 638-amino acid precursor to gp70 and p15E. In addition, there is a typical leader sequence preceding the amino terminus of gp70. The locations of potential glycosylation sites and other structural features indicate that the entire gp70 molecule and most of p15E are located on the outer side of the membrane. Internal cleavage of the env precursor to generate gp70 and p15E occurs immediately adjacent to several basic amino acids at the carboxyl terminus of gp70. This cleavage generates a region of 42 uncharged, relatively hydrophobic amino acids at the amino terminus of p15E, which is located in a position analogous to the hydrophobic membrane fusion sequence of influenza virus hemagglutinin. The mature polypeptides are predicted to associate with the membrane via a region of 30 uncharged, mostly hydrophobic amino acids located near the carboxyl terminus of p15E. Distal to this membrane association region is a sequence of 35 amino acids at the carboxyl terminus of the env precursor, which is predicted to be located on the inner side of the membrane. By analogy to Moloney murine leukemia virus, a proteolytic cleavage in this region removes the terminal 19 amino acids, thus generating the carboxyl terminus of p15E. This leaves 15 amino acids at the carboxyl terminus of p15E on the inner side of the membrane in a position to interact with virion cores during budding. The precise location and order of the large RNase T1-resistant oligonucleotides in the env region were determined and compared with those from several leukemogenic viruses of AKR origin. This permitted a determination of how the differences in the leukemogenic viruses affect the primary structure of the env gene products. (Author abstract) (54 Refs)

25. NUCLEOTIDE SEQUENCE OF THE TRANSFORMING GENE OF SIMIAN SARCOMA VIRUS.

Devare SG, Reddy EP, Robbins KC, Andersen PR, Tronick SR, Aaronson SA
Lab. Cellular and Molecular Biology, Natl. Cancer Inst., Bethesda, MD, 20205
Proc Natl Acad Sci USA; 79(10,part1):3179-3182 1982

The sequence of the transforming region of simian sarcoma virus (SSV) has been determined by using molecularly cloned viral DNA. This region encompassed the 1.0-kilobase pair woolly monkey cell-derived insertion sequence, v-sis, and flanking simian sarcoma-associated viral (SSAV) sequences. A 675-nucleotide-long open reading frame commenced 19 nucleotides within the SSAV sequences to the left of the v-sis helper viral junction and terminated within v-sis itself. Possible promoter and acceptor splice signals were detected in helper viral sequences upstream from this open reading frame, and potential polyadenylylation sites were identified downstream both within v-sis and in helper viral sequences beyond v-sis. The recombinational event that led to the generation of SSV occurred in the middle of two functional codons, indicating that SSAV provided the regulatory elements for transcription as well as the initiation codon for translation of SSV cell-derived transforming sequences. (Author abstract) (28 Refs)

26. NUCLEOTIDE SEQUENCE ANALYSIS OF THE LONG TERMINAL REPEAT OF INTEGRATED SIMIAN SARCOMA VIRUS: EVOLUTIONARY RELATIONSHIP WITH OTHER MAMMALIAN RETROVIRAL LONG TERMINAL REPEATS.

Devare SG, Reddy EP, Law JD, Aaronson SA
Lab. Cellular and Molecular Biology, NCI, Bethesda, MD, 20205
J Virol; 42(3):1108-1113 1982

Nucleotide sequence analysis of the long terminal repeat (LTR) of the integrated simian sarcoma virus showed that the simian sarcoma virus LTR comprised 504 nucleotides with an inverted repeat of seven bases at its 5' and 3' termini. At the site of simian sarcoma virus integration, cellular flanking sequences adjacent to the proviral LTR contained a direct repeat of four bases. A 13-base sequence after the 5' LTR was found to be complementary to prolyl tRNA, suggesting that tRNA-Pro may serve as the primer for reverse transcription of simian sarcoma virus RNA. The U5 and R regions, derived respectively from the 5' end and terminally redundant sequences of the viral RNA, were found to have similar organization and sequence homology close to that of Moloney murine sarcoma virus or Moloney murine leukemia virus. These results indicate that regions within LTRs with known functionally important sequences have been most well conserved during retrovirus evolution. (Author abstract) (45 Refs)

27. CONSERVATION OF PROTEIN CODING POTENTIAL IN THE LONG TERMINAL REPEATS OF EXOGENOUS AND ENDOGENOUS MOUSE MAMMARY TUMOR VIRUSES.

Peters G, Smith R, Brookes S, Dickson C
Imperial Cancer Res. Fund Lab., Lincoln's Inn Fields, London WC2A 3PX, England
J Virol; 42(3):880-888 1982

In vitro protein synthesis and DNA sequence analysis indicate that mouse mammary tumor virus differs from other well-characterized retroviruses in that the long terminal repeat region of the provirus has the capacity to encode proteins. Different exogenously transmitted mouse mammary tumor virus strains and endogenous proviral units conserved this open reading frame feature in the long terminal repeat despite a variation in nucleotide sequence. The proteins encoded by the different long terminal repeats were clearly related, but showed minor variations in size and tryptic peptide maps. In each case, the largest in vitro product had a mol wt of about 36,000 to 37,000, suggesting that the open reading frame sequences must extend for approx 1,000 nucleotides beginning at the extreme 5' end of the long terminal repeat. The fact that the reading frame was conserved among these viruses argues in favor of an in vivo function for the open reading frame protein. (Author abstract) (29 Refs)

28. NUCLEOTIDE SEQUENCE ANALYSIS OF THE LONG TERMINAL REPEAT OF AVIAN MYELOBLASTOSIS VIRUS AND ADJACENT HOST SEQUENCES.

Rushlow KE, Lautenberger JA, Reddy EP, Souza LM, Baluda MA, Chirikjian JG, Papas TS
(c/o Papas) Lab. Molecular Oncology, NCI, Bethesda, MD, 20205
J Virol; 42(3):840-846 1982

The nucleotide sequence of the integrated avian myeloblastosis virus long terminal repeat has been determined. The sequence is 385 base pairs long and is present at both ends of the viral DNA. The cell-virus junctions at each end consist of a six-base-pair direct repeat of cell DNA next to the inverted repeat of viral DNA. The long terminal repeat also contains promoter-like sequences, an mRNA capping site, and polyadenylation signals. Several features of this long terminal repeat suggest a structural and functional similarity with sequences of transposable and other genetic elements. Comparison of these sequences with long terminal repeats of other avian retroviruses indicates that there is a great variation in the 3' unique sequence (U3), whereas the 5' specific sequences (U5) and the R region are highly conserved. (Author abstract) (31 Refs)

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VIRAL PROTEINS

1. PROCESSING OF THE ENV GENE PRODUCTS OF MOLONEY MURINE LEUKEMIA VIRUS.

Ng VL, Wood TG, Arlinghaus RB
Dept. Tumor Virology, Univ. Texas at Houston, M. D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
J Gen Virol; 59(part2):329-343 1982

The initial env gene polyproteins present in Moloney murine leukemia virus (M-MuLV)-infected NIH/3T3 cells were examined to determine their relationship to each other as well as their role in generating env gene products gp70, p15(E) and p12(E). Steady-state labeling with [3H]fucose glucosamine revealed anti-gp69/71 immunoprecipitable proteins of mol wt 93,000 (gPr93env), 83,000 (gPr83env) and 70,000 (gp70), whereas similar labeling with [3H] showed only two bands of anti-gp69/71 immunoprecipitable radioactivity migrating in SDS-polyacrylamide gels with gPr93env and gp70. The gPr83env was the only polypeptide detected in 15 min [3H]leucine pulse-labelings. gPr83env, gp70, p15(E) and p12(E) were detected in pulse-chase experiments. Pretreatment of infected cells with tunicamycin, allowed the synthesis of a major band at mol wt 62,000 (Pr62env) and a minor band of 73,000 mol wt at the expense of gPr83 env. In pulse-chase experiments conducted in the presence of tunicamycin, Pr62env increased during the earl chase period but disappeared during the later stages of the chase. No product of Pr62env was detected. Cation-exchange chromatography of tryptic digests of radioactive tyrosine-labeled gPr83env, Pr62env and gp70 showed sequence relationships among the three proteins. Comparison of the two-dimensional fingerprints of [3H]leucine-labeled gPr83env and the mature proteins gp70 and p12(E) support their precursor-product relationship. gp70 and p12(E) seemed to share a few leucine-containing tryptic peptides. These results provide strong evidence that gPr83env is th primary product of the env gene which, upon tunicamycin treatment, is synthesized as a subglycosylated protein, Pr62env. It

HE 20.3172/2.

appears that gPr83env undergoes further modification of its core oligosaccharide structure to yield gPr93env. Our inability to detect gPr93env by [3H]leucine labelings suggests a close chronological relationship between fucosylation and cleavage of the precursor polyprotein, suggesting that cleavage of gPr93env yields gp70 and p15(E). The latter is further cleaved to yield p12(E) plus a polypeptide containing the C-terminal end of p15(E). (38 Refs)

2. MONOCLONAL ANTIBODIES TO THE P21 PRODUCTS OF THE TRANSFORMING GENE OF HARVEY MURINE SARCOMA VIRUS AND OF THE CELLULAR RAS GENE FAMILY.

Furth ME, Davis LJ, Fleurdelys B, Scolnick EM
Lab. Tumor Virus Genetics, NCI, Bethesda, MD, 20205
J Virol; 43(1):294-304 1982

We have isolated eight rat lymphocyte-myeloma hybrid cell line producing monoclonal antibodies that react with the 21,000-dalton transforming protein (p21) encoded by the v-ras gene of Harvey murine sarcoma virus (Ha-MuSV). These antibodies specifically immunoprecipitate both phosphorylated and non-phosphorylated forms of p21 from lysates of cells transformed by Ha-MuSV. All eight react with the products of closely related ras genes expressed in cells transformed by two additional sarcoma viruses (rat sarcoma virus and BALB sarcoma virus) or by a cellular Harvey-ras gene placed under the control of a viral promoter. Three of the antibodies also react strongly with the p21 encoded by the v-ras gene of Kirsten MuSV. These same three antibodies immunoprecipitate the predominant p21 species synthesized normally in a variety of rodent cell lines, including the p21 produced at high levels in 416B murine hemopoietic cells. This suggests that an endogenous gene closely related to Kirsten-ras is expressed in these cells. The monoclonal antibodies have been used to confirm two properties associated with p21: localization at the inner surface of the membrane of Ha-MuSV-transformed cells, assayed by immunofluorescence microscopy, and binding of guanine nucleotides. (Author abstract) (42 Refs)

3. A TEMPERATURE-SENSITIVE DEFECT IN THE EXPRESSION OF A SARCOMA VIRUS TRANSFORMING GENE.

Horn P, Janice J
The University of Texas Health Science Center at Houston Graduate School, of Biomedical Science Houston, TX
Diss Abstr Int [B]; 42(11):4397-B 1982

The viral proteins synthesized by a Moloney murine sarcoma virus (Mo-MuSV) with a temperature-sensitive mutation in a function required for the maintenance of the transformed state (ts110) were examined. Normal rat kidney cells (NRK) were infected with the ts110 virus and a non-virus-producing cell clone, termed 6m2, was isolated. This cell clone had a malignant phenotype at 33 degrees, the permissive temperature, but changed to a normal phenotype at 39 degrees. Two viral proteins were detected in 6m2 cells. A 58,000 dalton protein (P58) was detected at both 33 degrees and 39 degree and contained only core protein (gag) coded sequences. An 85,000 dalton protein (P85) was detected only at 33 degrees and contained sequences of viral core proteins p15, pp12, and part of p30 as well as protein sequences attributed by peptide mapping to P23 and P38, two candidate viral mouse src (v-mos) gene products. These results provide good evidence that P85 is a gag-mos polyprotein. As expected for a functional mos-gene product, P85 synthesis preceded parameters characteristic of the transformed state, including changes in cell morphology, in the cytoplasmic microtubule complex (CMTC) and in the rate of hexose uptake. (no Refs)

4. IDENTIFICATION OF PHOSPHOTYROSINE-CONTAINING PROTEINS IN UNTRANSFORMED AND ROUS SARCOMA VIRUS-TRANSFORMED CHICKEN EMBRYO FIBROBLASTS.

Martinez R, Nakamura KD, Weber MJ
Dept. Microbiology, Univ. Illinois, Urbana, IL, 61801
Mol Cell Biol; 2(6):653-665 1982

A methodology for identification of proteins which are phosphorylated on tyrosine residues by their mol wt is described. 32P-labeled whole cell lysates were electrophoresed through tubular polyarcylamide gels, and the phosphoamino acid content of the separated phosphoproteins was analyzed. Analysis of Rous sarcoma virus (RSV)-transformed chick embryo fibroblast cell lysates revealed a large number of phosphotyrosine-containing proteins encompassing a very wide range of mol wt (less than 20-greater than 200 kilodaltons). Application of the technique to untransformed cells indicated that the cells contain at least two major tyrosine-phosphorylated proteins. The transforming protein of RSV (pp60src) seems to directly or indirectly trigger a complex network of tyrosine phosphorylation events involving a large number of cellular proteins. The methodology presented is sufficiently sensitive to detect phosphotyrosine-containing proteins in cells characterized by low levels of total phosphotyrosine, such as untransformed cells. The method may prove useful for detecting minor tyrosine phosphorylation events that could occur after neoplastic transformation by agents that do not raise the overall phosphotyrosine level, such as papovaviruses, other retroviruses, and chemical carcinogens. (35 Refs)

VIRAL RNA

5. PARTICIPATION OF 5'-TERMINAL LEADER SEQUENCES IN IN VITRO TRANSLATION OF ROUS SARCOMA VIRUS RNA.

Perdue ML, Borchelt D, Resnick RJ
Div. Experimental Pathology, Dept. Pathology, Univ. Kentucky Medical Center, Lexington, KY, 40536
J Biol Chem; 257(11):6551-6555 1982

The cell-free translation of genome RNA from Rous sarcoma virus was examined following hybridization to selected fragments of viral DNA. Single-stranded fragments, generated by t-RNATrp primed transcription of 70 S RNA from the Schmidt-Ruppin D strain, were isolated and purified by electrophoresis. These included DNA complementary to the 5'-terminal 101 nucleotides (DNA100) of virion 38 S RNA and a collection of prematurely terminated transcripts which lack the complement to the extreme 5'-terminal 7-20 nucleotides (DNA less than 100). In addition, DNA encompassing the viral leader sequences was purified from a cloned copy of proviral DNA. Under hybrid-arrested translation conditions, the leader DNA as well as DNA100 inhibited translation of all protein products generated from the 70 S RNA, while hybridization to the shorter transcripts (DNA less than 100) did not affect in vitro protein synthesis. All single-stranded DNAs were shown to hybridize with equal efficiency to viral RNA under hybrid-arrested translation conditions and inhibition of protein synthesis by DNA100 was concentration-dependent. These results docu-

ment the participation of noncoding leader RNA in Rous sarcoma virus protein synthesis and demonstrate that a free, single-stranded 5' terminus is necessary for cell free translation of 70 S RNA. (Author abstract) (33 Refs)

6. ACUTE LEUKEMIA VIRUSES E26 AND AVIAN MYELOBLASTOSIS VIRUS HAVE RELATED TRANSFORMATION-SPECIFIC RNA SEQUENCES BUT DIFFERENT GENETIC STRUCTURES, GENE PRODUCTS, AND ONCOGENIC PROPERTIES.

Bister K, Nunn M, Moscovici C, Perbal B, Baluda MA, Duesberg PH
Max-Planck-Institut fur Molekulare Genetik, Ihnestrasse 63-73, D-1000 Berlin 33, W. Germany
Proc Natl Acad Sci USA· 79(12):3677-3681 1982

The genetic structure and gene products of E26 virus were analyzed for a comparison with those of AMV. An E26/helper virus complex was found to contain two RNA species: a 5.7-kilobase (kb) RNA than hybridizes with cloned AMV-specific proviral DNA and hence is probably the E26 genome; and an 8.5-kb RNA that is unrelated to AMV and represents helper virus RNA. Thus, E26 RNA is smaller than 7.5-kb AMV RNA. Hybridization of size-selected poly(A)-terminating E26 RNA fragments with AMV-specific DNA indicated that the shared specific sequences are located in the 5' half of the E26 genome as opposed to a 3' location in AMV RNA. In nonproducer cells transformed in vitro by E26, a gag-related nonstructural 135,000-dalton protein (p135) was found. No gag (Pr76) or gag-pol (Pr180) precursors of essential virion proteins, which are present in AMV nonproducer cells, were observed. p135 was also found in cultured E26 virus producing cells of several leukemic chickens, and its intracellular concentration relative to that of the essential virion proteins encoded by the helper virus correlates with the ratio of E26 to helper RNA in virions released by these cells. p135 is phosphorylated but not glycosylated and appears to be coded for by a partial gag gene and by E26-specific RNA sequences, presumably including those shared with AMV. Hence, AMV and E26 appear to use different strategies for the expression of related onc sequences: AMV is thought to encode a transforming protein via a subgenomic mRNA, wherease E26 codes for a gag-related polyprotein via genomic RNA. It is speculated that differences in the oncogenic properties of E26 and AMV are due to differences in their genetic structures and gene products. (32 Refs)

7. TRANSCRIPTS FROM THE CELLULAR HOMOLOGS OF RETROVIRAL ONCOGENES: DISTRIBUTION AMONG CHICKEN TISSUES.

Gonda TJ, Sheiness DK, Bishop JM
Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143
Mol Cell Biol; 2(6):617-624 1982

RNA transcribed from each the four c-onc genes c-myb, c-myc, c-erb, and c-src was quantified in a variety of tissues and cells by dot-blot assay with standardized avian myeloblastosis virus (AMV) 70S virion RNA. Cells examined for levels of c-onc RNAs included fibroblasts, whole embryo, yolk sac, Marek's disease-transformed cells (MSB-1), avian erythroblastosis virus (AEV) erythroblasts and fibroblasts and AMV myeloblasts. Transcription from each c-onc gene was detected (as c-myc, c-erb, and c-src RNA) in nearly all cells and tissues examined, but significant c-myb transcription was detected only in hemopoietic cells. The amount of c-onc RNAs varied, relatively little variation was observed in c-erb levels, a greater range in c-myc and c-src RNA levels was noted, and the greatest variations were exhibited by c-myb levels. Organs expressing the highest levels of c-myb (bone marrow, yolk sac, thymus) are major hemopoietic organs of the chicken, suggesting that c-myb may be expressed in immature hemopoietic cells. The high level of c-myb expression found in AEV-transformed erythroblasts suggests that c-myb function is not restricted to myelomonocytic cells. No obvious correlation existed between susceptibility to transformation and c-onc expression. (40 Refs)

8. EXPRESSION OF EUKARYOTIC VIRAL AND CELLULAR GENES.

Pettersson RF, Kaariainen L, Soderlund H, Oker-Blom N
Dept. Virology, Univ. Helsinki, Helsinki, Finland
Expression of Eukaryotic Viral and Cellular Genes. London, Academic Press, 324 pp., 1981.

Recent advances in molecular virology, molecular genetics, and recombinant DNA research are combined in a discussion of expression of eukaryotic viral and cellular genes. Structure and expression of DNA virus genes are reviewed with respect to molecular analysis of adenovirus transformation, control of adenovirus messenger (m)RNA production by differential transcription and differential stability of mRNA, an early adenovirus glycoprotein and its association with transplantation antigens, the control of adenovirus early gene expression, and mutational analysis of regulatory elements of simian virus 40. Retrovirus genes are discussed with respect to OK 10 virus-transformed bone-marrow-derived cell line, the transforming insert in the Abelson murine leukemia virus genome, and DNA intermediates in the replication of retroviruses. RNA virus genes are discussed with respect to the genome of single-stranded messenger-sense RNA viruses, influenza virus, capped cellular RNAs, drift and shift of influenza A-type virus, vesicular stomatitis virus and host cell phosphoproteins, initiation of eukaryotic protein synthesis, alphavirus gene products, Semliki Forest virus, RNA containing heterogeneous 5'-terminal nucleotide sequences, and Semliki Forest virus defective interfering RNA using DNA copies. Eukaryotic genes are discussed with respect to structure, organization and developmental expression of chorion multigene families, gene amplification and methotrexate resistance, RNA-protein complexes precipitated by Lupus antibodies, promoter sequences, expression of cloned eukaryotic genes in microorganisms, human genomic DNA-encoding Pro-opiomelanocortin, and functional yeast minichromosomes containing centromeric DNA. (701 Refs)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

9. SYNTHESIS OF MURINE LEUKEMIA VIRUS PLUS STRONG STOP DNA INITIATES AT A UNIQUE SITE.

Mitra SW, Chow M, Champoux J, Baltimore D
Dept. Biochemical Genetics, Merck Sharp and Dohme Res. Lab., Rahway, NJ, 07065
J Biol Chem; 257(11):5983-5986 1982

The 5'-terminal segment of plus strong stop DNA has been isolated from an endogenous virion reverse transcription reaction and characterized. The plus strong stop DNA is shown to initiate with the sequence 5'AATGAAAGA. The 5'-terminal nucleotide is a deoxyribonucleotide. No residual protein or RNA moiety was found attached to the plus strong stop DNA.

Thus, the mechanism which primes plus strand synthesis is still unclear. No evidence for multiple initiation sites or heterogeneous starts is observed. This data indicates that the initiation of plus strong stop DNA synthesis by the virion RNA-dependent DNA polymerase is a precise event which occurs at a unique site on the retrovirus genome. (Author abstract) (19 Refs)

10. STUDIES WITH APHIDICOLIN ON THE FV-1 HOST RESTRICTION OF FRIEND MURINE LEUKEMIA VIRUS.

Chinsky J, Soeiro R
Dept. Medicine, Microbiology, Cell Biology, Albert Einstein Coll. Medicine, Bronx, NY, 10461
J Virol; 43(1):182-190 1982

The murine gene Fv-1 exerts a major control over the replication of Friend murine leukemia virus (F-MuLV). An effect of the gene product has been determined to be at the level of accumulation and integration of viral DNA. Aphidicolin, an inhibitor of eucaryotic DNA polymerase alpha, was studied in murine cells infected either permissively or nonpermissively with regard to the Fv-1 genotype. Results indicated that inhibition of DNA polymerase alpha did not affect the accumulation of Form III viral DNA in either permissive or nonpermissive cells. However, the normal accumulation of circular Form I DNA in permissive cells was inhibited. The block in the accumulation of Form I DNA resembled that occurring in some F-MuLV Fv-1-nonpermissive infections. Additionally, aphidicolin treatment resulted in the accumulation of novel low-molecular-weight viral DNA species, normally detectable in a nonpermissive infection of NIH cells with B-tropic F-MuLV. These data suggest that the Fv-1 gene product may interact with host DNA polymerase alpha to prevent viral replication. (Author abstract) (25 Refs)

11. SEQUENCE OF THE LONG TERMINAL REPEAT AND ADJACENT SEGMENTS OF THE ENDOGENOUS AVIAN VIRUS ROUS-ASSOCIATED VIRUS 0.

Hughes SH
Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724
J Virol; 43(1):191-200 1982

An infectious unintegrated circular Rous-associated virus (RAV-0) DNA was molecularly cloned, and the sequence of the long terminal repeat (LTR) and adjacent segments was determined. The sequence of the LTR was found to be very similar to that of replication-defective endogenous virus EV-1. Like the EV-1 LTR, the RAV-0 LTR is smaller than the LTRs of the oncogenic members of the avian sarcoma virus-avian leukosis virus group. There is, however, significant homology. The most striking differences are in the U3 region of the LTR, and in this region there are a series of small segments present in the oncogenic viruses which are absent in RAV-0. These differences in the U3 region of the LTR could account for the differences in the oncogenic potential of RAV-0 and the avian leukosis virus. The regions adjacent to the RAV-0 LTR were compared with the available avian sarcoma virus sequences. A segment of approx 200 bases to the right of the LTR (toward gag) is almost identical in RAV-0 and the Prague C strain of Rous sarcoma virus. The segment of RAV-0 which lies between the end of the env gene and U3 is approx 190 bases in length. Essentially this entire segment is present between env and src in the Schmidt-Ruppin A strain of Rous sarcoma virus. Most of this segment is also present between env and src in Prague C; however, in Prague C there is an apparent deletion of 40 bases in the region adjacent to env. In Schmidt-Ruppin A, but not in Prague C, about half of this segment is also present between src and the LTR. This arrangement has implications for the mechanism by which src was acquired. The region which encoded the gp37 portion of env appears to be very similar in RAV-0 and the Rous sarcoma viruses. However, differences at the very end of env imply that the carboxy termini of RAV-0, Schmidt-Ruppin A, and Prague C gp37s are significantly different. The implications of these observations are considered. (30 Refs)

12. ENVELOPE GENE SEQUENCES WHICH ENCODE THE GP52 PROTEIN OF SPLEEN FOCUS-FORMING VIRUS ARE REQUIRED FOR THE INDUCTION OF ERYTHROID CELL PROLIFERATION.

Linemeyer DL, Menke JG, Ruscetti SK, Evans LH, Scolnick EM
Dept. Biochemical Genetics, Merck Res. Lab., Rahway, NJ, 07065
J Virol; 43(1):223-233 1982

A series of insertion-deletion mutants was constructed in a molecularly cloned DNA copy of the Friend strain of spleen focus-forming virus (SFFV). The mutants were produced by inserting a synthetic oligonucleotide linker containing the recognition sequence of SalI endonuclease into several different locations of the SFFV DNA. Three classes of mutants were isolated: insertion-deletion mutants in the 5' half of the SFFV genome, in the long terminal repeat of the SFFV genome, and in the env gene of the SFFV genome. The env gene mutant has a deletion of sequences shared in common between the env gene of SFFV and the env genes of mink cell focus-inducing murine leukemia viruses. From analyses of the biological activity of the various mutants and a biologically active subgenomic SFFV DNA fragment described herein, we can deduce that the coding sequence encompassing the env gene of SFFV is required for the biological activity. This region, required for the pathogenic phenotype, cannot be larger than 1.5 kilobase pairs, a size only slightly more than that sufficient to encode the nonglycosylated precursor of the gp52 env gene product. (Author abstract) (45 Refs)

13. INTERNAL ORGANIZATION OF ENDOGENOUS PROVIRAL DNAS OF XENOTROPIC MURINE LEUKEMIA VIRUSES.

Hoggan MD, Buckler CE, Sears JF, Chan HW, Rowe WP, Martin MA
Lab. Viral Diseases, Natl. Inst. Allergy and Infectious Diseases, Bethesda, MD, 20205
J Virol; 43(1):8-17 1982

The internal organization of endogenous xenotropic murine leukemia virus proviruses was determined in a series of blot hybridization experiments in which DNA from several different inbred mouse strains, digested with restriction enzymes known to cleave xenotropic proviral DNAs at least twice, was annealed to generalized murine leukemia virus or xenotropic env-specific DNA probes. Comigrating bands of variable intensity which hybridized to the xenotropic env probe were identified in all inbed mouse DNA preparations. At least seven classes of endogenous xenotropic proviral DNA with respect to SacI cleavage maps were detected in mouse DNA. Two of the seven classes were indistinguishable from proviruses associated with known infectious xenotropic murine leukemia viruses. These results are consistent with the existence of related but organizationally distinct families of endogenous xenotropic proviral DNA that are present in different relative abundances in mouse genomic DNA. (Author abstract) (20 Refs)

14. MOLECULAR CLONING OF AKR XENOTROPIC MURINE LEUKEMIA VIRUS UNINTEGRATED PROVIRAL DNA.

Joseph DR
Robert V. Slater Lab., Papanicolaou Cancer Res. Inst., 1155 N.W. 14th St., Miami, FL, 33136
Gene; 17(3):341-344 1982

Cloning of AKR xenotropic murine leukemia virus (MuX) superhelical proviral DNA from infected mink cells is described. Superhelical DNA was purified from Hirt supernatant extracts after electrophoresis on low-melting-point agarose gels. Purified BamHI-treated proviral DNA was ligated with Charon 28 DNA, the recombinant DNA was packaged in vitro, and the resulting phage were plated on DP50supF. Of the 1,000 plaques that were screened for viral DNA, approx 550 contained non-lambdoid DNA inserts and nine of these phage contained MuX DNA. Gel electrophoresis and Southern blotting analyses of the nine recombinants revealed that seven had inserts of 9.0 kilobase (kb) DNA and two contained inserts of less than full-length 9.0 kb DNA. Restriction endonuclease analyses revealed that six of the 9.0 kb inserts had restriction maps identical to full-length viral DNA with tandem LTR units. One isolate contained a 2.3 kb inversion around one LTR in the envelope region. One insert contained a deletion of 0.45 kb in one 0.6 kb LTR unit and another insert contained a deletion of one LTR unit and adjacent 0.2 kb DNA in the gag-gene region. The inverted DNA was in a similar position as the inversion isolate (2.3-2.4 kb from the LTR junction into the envelope gene). (26 Refs)

15. IDENTIFICATION OF A DNA FRAGMENT FROM A MOLECULARLY CLONED MINK CELL FOCUS-INDUCING MURINE LEUKEMIA VIRUS SPECIFIC FOR XENOTROPIC VIRUS-RELATED SEQUENCES.

Yoshimura FK
Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
J Virol; 43(1):348-351 1982

Molecular clones of closed circular DNA molecules of a mink cell focus-inducing murine leukemia virus (MCF-13 MuLV) were generated. Closed circular DNA molecules isolated from a Hirt extraction of recently infected NIH/3T3 cells were inserted at their unique EcoRI site into lambdagtWES-lambdaB. Restriction endonuclease analysis of inserts of two clones indicated that they represented intact MCF-13 MuLV genomes. One viral insert contained two large terminal repeat sequences, and the other contained only one. A 300-base-pair DNA fragment located in the envelope region of the MCF-13 MuLV genome was determined to be related to xenotropic MuLV sequences. (Author abstract) (19 Refs)

16. MOLECULAR CLONING AND CHARACTERIZATION OF MURINE LEUKEMIA VIRUS-RELATED DNA SEQUENCES FROM C3H/HEN MOUSE DNA.

Roblin R, Young JM, Mural RJ, Bell TE, Ihle JN
Cancer Biology and Biological Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD, 21701
J Virol; 43(1):113-126 1982

Ten murine leukemia virus (MuLV)-related DNA sequences were isolated from C3H/HeN mouse genomic DNA by cloning of EcoRI fragments in a Charon 4A vector. Detailed restriction endonuclease maps of four of the clones were developed by using AKR MuLV [32P]cDNA as a probe. C3H clone 14-9 contains approx seven kilobase pairs of MuLV-related DNA, one copy of an MuLV long terminal repeat-like sequence, and a region of flanking mouse DNA. C3H clones 34.2 and 36.1 contain approximately two kilobase pairs of MuLV-related DNA, one copy of a MuLV LTR-like sequence, and differing lengths of flanking mouse DNA sequences. C3H clone 8.13 was found to contain an insert of 5.7 kilobase pairs of MULV-related DNA with two long terminal repeat-like regions and sequences which are partially homologous to AKv-1. Comparison of the restriction endonuclease cleavage maps of these C3H clones with maps recently developed for ecotropic and xenotropic MuLV DNAs indicates that C3H clone 14-9 corresponds to the 5'-terminal portion of a genomic DNA sequence related to xenotropic MuLVs, whereas C3H clones 34.2 and 36.1 correspond to the 3' terminal portions of genomic DNA sequences related to xenotropic MuLVs. Clone 8.13 represents a deleted, xenotropic MuLV-related provirus. C3H clones 14-9, 34.2, 36.1, and 8.13 provide defined DNA sequence probes with which to characterize the organization and expression of endogenous MuLV-related DNA sequences in the mouse genome. (Author abstract) (31 Refs)

17. MOST OF THE MURINE LEUKEMIA VIRUS SEQUENCES IN THE DNA OF NIH/SWISS MICE CONSIST OF TWO CLOSELY RELATED PROVIRUSES, EACH REPEATED SEVERAL TIMES.

Steffen DL, Mural R, Cowing D, Mielcarz J, Young J, Roblin R
Worcester Foundation for Experimental Biology, Shrewsbury, MA, 01545
J Virol; 43(1):127-135 1982

The structure of the endogenous murine leukemia virus (MuLV) sequences of NIH/Swiss mice was analyzed by restriction endonuclease digestion, gel electrophoresis, and hybridization to an MuLV nucleic acid probe. Digestion of mouse DNA with certain restriction endonucleases revealed two classes of fragments. A large number of fragments (about 30) were present at a relatively low concentration, indicating that each derived from a sequence present once in the mouse genome. A smaller number of fragments (1 to 5) were present at a much higher concentration and must have resulted from sequences present multiple times in the mouse genome. These results indicated that the endogenous MuLV sequences represent a family of dispersed repetitive sequences. Hybridization of these same digested mouse DNAs to nucleic acid probes representing different portions of the MuLV genome allowed construction of a map of the sites where restriction endonucleases cleave the endogenous MuLV sequences. Several independent recombinant DNA clones of endogenous MuLV sequences have been isolated from C3H mice. Analysis of these sequences shows that they have the structure of MuLV proviruses. The sites at which restriction endonucleases cleave within these proviruses appeared to be similar or identical to the sites at which these nucleases cleaved within the MuLV sequences of NIH/Swiss mice. This identity was confirmed by parallel electrophoresis. We conclude that the apparently complex pattern of endogenous MuLV sequences of NIH/Swiss mice consists largely of only two kinds of provirus, each repeated multiple times at dispersed sites in the mouse genome. (Author abstract) (15 Refs)

18. NUCLEOTIDE SEQUENCE OF THE TRANSFORMING GENE OF AVIAN MYELOBLASTOSIS VIRUS.

Rushlow KE, Lautenberger JA, Papas TS, Baluda MA, Perbal B, Chirikjian JG, Reddy EP
(c/o Papas) Lab. Molecular Oncology, NCI, Bethesda, MD, 20205
Science; 216(4553):1421-1423 1982

Avian myeloblastosis virus is defective in reproductive capacity, requiring a helper virus to provide the viral proteins essential for synthesis of new infectious virus. This virus arose by recombination of the nondefective helper virus and host cellular sequences present within the normal avian genome. These latter sequences are essential for leukemogenic activity. The complete nucleotide sequence of this region is reported. Within the acquired cellular sequences there is an open reading frame of 795 nucleotides starting with the initiation codon ATG (adenine, thymine, guanine) and terminating with the triplet TAG. This open reading frame could code for the putative transforming protein of 265 amino acids with a mol wt of approx 30,000. (Author abstract) (17 Refs)

19. MOLECULAR CLONING AND SEQUENCE DETERMINATION OF A MURINE SARCOMA VIRUS GENOME (MEETING ABSTRACT).

Van Beveren C, Van Straaten F, Papkoff J, Lai MH, Muller R, Hunter T, Verma IM
The Salk Institute, P. O. Box 85800, San Diego, CA, 92138
In Vitro; 18(3,part2):314 1982

Moloney murine sarcoma virus (Mo-MSV), isolated from a Moloney murine leukemia virus (Mo-MLV)-induced mouse rhabdomyosarcoma, is a helper-dependent, C-type retrovirus which elicits the rapid appearance of sarcomas at the site of injection in mice. The analysis of its genome indicated that the 5' half was essentially the same as that of helper Mo-MLV, whereas the 3' end contained a major substitution of cellular DNA. The amount of viral transforming product(s) obtained in infection was so low as to preclude detection by standard means, and no tumor-specific antiserum was produced in tumor-bearing animals. Upon molecular cloning of infectious, unintegrated Mo-MSV double-stranded DNA genomes, the determination of the nucleotide sequence of the 5833-nucleotide genome was greatly facilitated. It was then possible to predict the amino acid sequence of the product of the transforming v-mosMo gene. Peptides corresponding to the N- and C- termini of the putative protein were synthesized and used to raise antibodies in rabbits. Immunoprecipitation with sera against the 12-amino acid C-terminus permitted the identification of a 37kd product of the v-mosMo gene in acutely infected NIH-3T3 cells. The product exists in two forms which differ in degree of phosphorylation. No protein kinase activity has been demonstrated to be associated with either form. (no Refs)

20. ORGANIZATION, DISTRIBUTION, AND STABILITY OF ENDOGENOUS ECOTROPIC MURINE LEUKEMIA VIRUS DNA SEQUENCES IN CHROMOSOMES OF MUS MUSCULUS.

Jenkins NA, Copeland NG, Taylor BA, Lee BK
Jackson Lab., Bar Harbor, ME, 04609
J Virol; 43(1):26-36 1982

The endogenous ecotropic murine leukemia virus DNA content and integration sites were characterized for 54 inbred strains and substrains of mice by restriction enzyme digestion, Southern blotting, and hybridization with an ecotropic murine leukemia virus DNA-specific probe. More than 75% of these strains carried endogenous ecotropic proviruses which were located in at least 29 distinct integration sites in chromosomes of Mus musculus. Fourteen of these proviruses have been assigned specific locus designations. Most, but not all, of the endogenous ecotropic proviruses were structurally indistinguishable by this analysis from the prototype AKR ecotropic virus, and the distribution of these proviruses followed known relationships among the inbred strains and substrains of mice. These results suggest that, in general, viral DNA integration preceded the establishment of inbred mouse strains and that these integrations are relatively stable. (Author abstract) (42 Refs)

21. IDENTIFICATION OF A UNIQUE MOUSE MAMMARY TUMOR VIRUS IN THE BALB/CNIV MOUSE STRAIN.

Puma JP, Fanning TG, Young LJ, Cardiff RD
Chiron Corporation, Berkeley, CA, 95616
J Virol; 43(1):158-165 1982

We examined the genetic structure, in terms of restriction endonuclease recognition sites, of the milk-transmitted, low-oncogenic mouse mammary tumor virus (MuMTV) of the BALB/cNIV mouse strain. An analysis with EcoRI documented the presence of acquired cNIV proviruses in the mammary tumor DNAs of BALB/cNIV animals. A comparison of tumor DNAs digested with PstI showed that both the cNIV MuMTV and C3Hf MuMTV proviruses lacked the 4.3-and 1.1-kilobase pair fragments characteristic of C3H MuMTV patterns. An examination of mammary tumor and normal, non-mammary tissue DNAs with BamHI supported the idea that the cNIV MuMTV is identical to the C3Hf MuMTV and demonstrated that these two low-oncogenic proviruses are identical to the high-oncogenic C3H MuMTV provirus with respect to a pair of BamHI sites which define a 1.3-kilobase pair fragment. For each of the three MuMTV strains, we also mapped DNAs generated in isolated virions by reverse transcription of their genomic RNAs. Our results showed that cNIV and C3Hf MuMTV are distinct entities by virtue of an additional PstI site within the cNIV long terminal repeat sequence. Another unique feature of cNIV MuMTV revealed by the analysis of virion-generated DNAs was the existence of a family of genomes within the cNIV population. We concluded that cNIV is distinct from its presumptive C3Hf MuMTV predecessor. (Author abstract) (26 Refs)

22. LOSS OF VIRAL GENE EXPRESSION AND RETENTION OF TUMORIGENICITY BY ABELSON LYMPHOMA CELLS.

Grunwald DJ, Dale B, Dudley J, Lamph W, Sugden B, Ozanne B, Risser R
McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
J Virol; 43(1):92-103 1982

Lymphomas induced by the Abelson murine leukemia virus (A-MuLV) were examined for the expression of biochemical and biological markers associated with A-MuLV transformation before and after in vivo growth in genetically distinguishable host mice. Although all tumors and clonal lines derived from them initially expressed the A-MuLV-encoded gag fusion protein p160, they ceased synthesis of this molecule after several weeks of growth in vivo as ascites tumors. Transplanted clonal lines continued to express the alloantigenic marker H-2b and the isoenzyme marker Gpi-1b of the donor tumor cells, indicating that the cells were of donor and not host origin. Examination of cellular DNA obtained from p160-positive and derivative p160-negative lines indicated that p160-negative

clones had lost A-MuLV-specific proviral sequences as detected by hybridization with several probes. Although the clonal lines no longer expressed p160, they retained their malignant phenotype and continued to express the Abelson antigen, a cell surface marker associated with A-MuLV lymphomagenesis. Continued expression of the A-MuLV genome was not required for maintenance of oncogenic potential under these conditions of in vivo tumor growth. (Author abstract) (38 Refs)

23. TUMORIGENIC TRANSFORMATION OF MAMMALIAN CELLS INDUCED BY A NORMAL HUMAN GENE HOMOLOGOUS TO THE ONCOGENE OF HARVEY MURINE SARCOMA VIRUS.

Chang EH, Furth ME, Scolnick EM, Lowy DR
Dept. Pathology, Uniformed Services Univ. of Health Sciences, Bethesda, MD, 20014
Nature; 297(5866):479-483 1982

A normal human gene homologous to the p21 ras oncogene of Harvey murine sarcoma virus induced oncogenic transformation and high p21 ras levels in murine fibroblasts when this gene was ligated to a control element (the long terminal repeat) from a murine or feline retrovirus. These results indicate that high levels of a gene product encoded by a normal human oncogene can induce tumorigenic transformation. (Author abstract) (37 Refs)

24. HUMAN EJ BLADDER CARCINOMA ONCOGENE IS HOMOLOGUE OF HARVEY SARCOMA VIRUS RAS GENE.

Parada LF, Tabin CJ, Shih C, Weinberg RA
Center for Cancer Res., Dept. Biology, Massachusetts Inst. Technology, Cambridge, MA, 02139
Nature; 297(5866):474-478 1982

Examination of homologies between retroviral oncogenes and transforming sequences defined by transfection reveals that the human bladder carcinoma (EJ) oncogene is homologous to the Harvey sarcoma virus oncogene (ras). Structural analysis limits the region of homology to a 3.0-kilobase SacI fragment of the EJ oncogene. Both EJ and ras DNA probes detect similar transcripts in transfectants derived from bladder carcinoma cell lines. (Author abstract) (42 Refs)

25. TRANSFORMING GENES OF HUMAN BLADDER AND LUNG CARCINOMA CELL LINES ARE HOMOLOGOUS TO THE RAS GENES OF HARVEY AND KIRSTEN SARCOMA VIRUSES.

Der CJ, Krontiris TG, Cooper GM
Sidney Farber Cancer Inst., Boston, MA, 02115
Proc Natl Acad Sci USA; 79(11):3637-3640 1982

Blot hybridization analysis indicated that NIH 3T3 mouse cells transformed by high mol wt DNAs of a human bladder and a human lung carcinoma cell line contained new sequences homologous, respectively, to the transforming genes of Harvey (rasH) and Kirsten (rasK) sarcoma viruses. The unique ras sequences were present in multiple independent NIH cell lines transformed in both primary and secondary transfection assays and corresponded to ras sequences normally present in human DNAs. The ras gene product was expressed in NIH cells transformed by bladder carcinoma DNAs and in the human bladder carcinoma cell lines at levels two- to four-fold greater than the level observed in nontransformed NIH 3T3 cells. These results indicate that the transforming genes of these human tumor cell lines are the cellular homologs of two retroviral transforming genes. (Author abstract) (30 Refs)

26. THE ONCOGENIC CIRCLE CLOSES.

Rigby PW
Imperial Coll. Science and Technology, London SW7 2BZ, England
Nature; 297(5866):451-453 1982

Characterization of cellular oncogenes is reviewed. Oncogenes exhibit target specificity within a lineage. Recombinant DNA technics have been applied to the cloned human bladder carcinoma oncogene. The relationship between cellular oncogenes and proto-oncogenes (genes detected by transfection) has been studied. Retrovirus onc genes have been cloned and used to analyze DNA of NIH cells transformed by a variety of cellular oncogenes. The cloned human bladder carcinoma oncogene has been shown to be homologous to ras, the transforming gene of Harvey murine sarcoma virus. Mechanisms of oncogene activation and action are discussed. (19 Refs)

27. GENE AMPLIFICATION.

Schimke RT
Dept. Biological Sciences, Stanford Univ., Stanford, CA
Gene Amplification. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, 339 pp., 1982.

Gene duplication and amplification studies are reviewed and extended abstracts of a conference on gene amplification are presented. Examples of gene amplification include dihydrofolate reductase gene amplification in murine and Chinese hamster ovary cell lines; in a methotrexate-resistant human leukemia cell line (K562); in rat hepatoma cells; in antifolate-sensitive and -resistant Chinese hamster lung cells; in antifolate-resistant, vincristine-resistant, and human neuroblastoma cells; in reciprocally cross-resistant uptake mutants of neuroblastoma; and in vincristine-resistant Chinese hamster ovary cells. Phenotypic reversion as a consequence of gene amplification, altered regulation of the mouse metallothionein-I gene, amplification of the gene for asparagine synthetase, and control of DHFR DNA replication and messenger RNA production. Other aspects of gene amplification are discussed, including studies involving the SEWA mouse tumor, clonogenic human tumor cells, human small cell lung cancer, Drosophila, and chicken actin genes during myogenesis. Molecular structures of amplified DNA structures in mouse cell lines and Chinese hamster ovary cells are described and methods for cloning amplified DNA, analysis of CAD gene amplification, and cloning and analysis of double minute DNA are reviewed. Mechanisms of gene amplification are proposed. Chromosomal alterations are discussed with respect to nonrandom gain of chromosomes in human malignant hematologic diseases, a chromosome defect in most cancers, the biologic role for chromatid exchange in mammalian somatic cells, and a common denominator in the tumorigenic action of viral oncogenes and nonrandom chromosomal changes. (295 Refs)

28. ROLE OF METHYLATION IN THE INDUCED AND SPONTANEOUS EXPRESSION OF THE AVIAN ENDOGENOUS VIRUS EV-1: DNA STRUCTURE AND GENE PRODUCTS.

Conklin KF, Coffin JM, Robinson HL, Groudine M, Eisenman R
Dept. Molecular Biology and Microbiology, Tufts Univ. Sch. Medicine, Boston, MA, 02111
Mol Cell Biol; 2(6):638-652 1982

A variant chicken embryo (number 1836) cell culture was examined by immunoprecipitation and gel electrophoresis. The culture contained endogenous avian provirus ev-1 and ev-6, and spontaneously expressed the ev-1 provirus. The cells released noninfectious virions containing Rous-associated virus

type O-related 70S RNA and gag gene products but devoid of reverse transcriptase activity or antigens. EV-1 encoded particles also lacked envelope glycoproteins. DNA from the spontaneously expressing 1836 cell ev-1 provirus contained deoxyribonuclease I-hypersensitive sites in both long-term repeat sequences and the 5' flanking and gag gene sequences, the DNA also contained a pattern of methylation different from that of inactive ev-1 DNA. The finding of specific sites in 1836 ev-1 DNA that are differentially methylated only in expressed ev-1 DNA suggests that a certain pattern of methylation, possibly undermethylation, effects transcriptional activation. (50 Refs)

29. UNUSUAL METHYLATION PATTERN OF THE ALPHA2(I) COLLAGEN GENE.

McKeon C, Ohkubo H, Pastan I, De Crombrugghe B
Lab. Molecular Biology, Div. Cancer Biology and Diagnosis, NCI, NIH, Bethesda, MD, 20205
Cell; 29(1):203-210 1982

We studied the methylation pattern of the alpha2 (Type I) collagen gene in DNA from five cell types with varying rates of Type I collagen synthesis: chick embryo fibroblast (CEF), CEF transformed by Rous sarcoma virus, erythrocyte, brain and sperm. The methylation-sensitive restriction enzymes, Msp I, Hpa II, Ava I and Sma I were used to detect methylation in three regions of the alpha2 (Type I) collagen gene: a 5.7 kb region, which includes the start site of transcription and the first two exons of the collagen gene; a 5.2 kb region containing exons in the middle of the gene; and a 3.5 kb region containing exons in the 3' portion of the gene. The DNA around the start site of transcription is not methylated whether or not the cells synthesize collagen. In contrast, the DNA from the central and 3' region of the gene is methylated to about the same extent whether or not the cells make collagen. Our data indicate that a gene that is methylated can be actively transcribed and that the level of expression of the alpha2 (Type I) collagen gene seems to be independent of methylation. (Author abstract) (36 Refs)

ANTIVIRALS

30. INHIBITION OF PHORBOL ESTER ENHANCEMENT OF MOUSE MAMMARY TUMOR VIRUS PRODUCTION BY ANTI-GLUCOCORTICOIDS (MEETING ABSTRACT).

Chan R
Department of Biomedical and Environmental Health Sciences, University of California, Berkeley, CA, 94720
In Vitro; 18(3,part2):309 1982

Production of mouse mammary tumor virus (MMTV) is known to be significantly enhanced by glucocorticoid hormones in infected cell cultures. More recently, it has been shown that the tumor promoting phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), can also stimulate MMTV production. In this investigation, four compounds, deoxycorticosterone, 11-deoxycortisol, 17alpha-methyltestosterone and progesterone were shown to be have anti-glucocorticoid activity as determined by their ability to antagonize the stimulation of MMTV production by dexamethasone in Mm5mt cells. All four of these anti-glucocorticoids also proved to be effective in antagonizing TPA-induced enhancement of MMTV production, as measured by a RNA-dependent DNA polymerase assay. (no Refs)

31. INHIBITION OF MURINE LEUKAEMIA VIRUS REVERSE TRANSCRIPTASE BY 2-HALOGENATED POLYADENYLIC ACIDS.

Fukui T, De Clercq E
Faculty Pharmaceutical Sciences, Osaka Univ., 133-1 Yamada-Kami, Suita, Osaka, Japan
Biochem J; 203(3):755-760 1982

Several new analogues of polyadenylic acid [(A)n], ie poly(2-fluoroadenylic acid) [(fl2A)n], poly(2-chloroadenylic acid) [(cl2A)n], poly(2-bromoadenylic acid) [(br2A)n] and poly(2-iodoadenylic acid) [(io2A)n] have been synthesized and evaluated for their effects on the RNA-directed DNA polymerase (reverse transcriptase) activity of Moloney murine leukemia virus. All (A)n analogues were found to be potent inhibitors of reverse transcriptase, the order of (decreasing) potency being (fl2A)n greater than (io2A)n greater than (br2A)n greater than (cl2A)n. For all four (A)n analogues the inhibition of reverse transcriptase was competitive with respect to the template-primer, (A)n-oligo(dT). The Ki values were 0.02 ug/ml for (fl2A)n, 0.1 ug/ml for (io2A)n, 0.5 ug/ml for (br2A)n and 8 ug/ml for (cl2A)n. With a Ki of 0.02 ug/ml (approx 0.04 uM), (fl2A)n can be considered as one of the most, if not the most, potent polynucleotide inhibitor of reverse transcriptase that has been described so far. (Author abstract) (22 Refs)

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VIRAL PROTEINS

1. RADIOIMMUNOASSAY FOR MAJOR EXTERNAL GLYCOPROTEIN OF MURINE MAMMARY TUMOR VIRUS.

Chiplunkar SV, Karande KA
Dept. Immunology, Cancer Res. Inst., Bombay 400 012, India
Indian J Exp Biol; 20(2):121-125 1982

A radioimmunoassay assay is described in which highly purified intact murine mammary tumor virus (MuMTV) virions from the expressed milk of strains C3H(Jax) and ICRC mice were externally labeled with 125I in the presence of lactoperoxidase. Heterologous polyvalent antisera prepared against disrupted virions were used for immunoprecipitation. Sodium dodecyl sulfate polyacrylamide electrophoresis showed that both iodinated virions migrated as a single peak with an estimated mol wt of 49,000 daltons. This peak corresponds to the previously described major membrane glycoprotein (gp 49) of MuMTV. Competition radioimmunoassays using antisera to MuMTV C3H and MuMTV ICRC with their respective labeled virions were shown to be specific for MuMTV antigens. Tissue extracts of C57BL liver and lactating mammary gland, ICRCf liver, swiss mouse milk plasma and fetal calf serum proteins did not compete in the assay. The addition of 4 ug of unlabeled MuMTV ICRC completely inhibited the binding of anti-MuMTV ICRC serum to 125I-labeled MuMTV ICRC. Large amounts of gp 49 were found in tumor extracts (940-3,937 nanog/mg) and sera (160-680 nanog/ml) of C3H and ICRC tumor-bearing mice. The assay can detect 3 nanog of antigen and is highly reproducible. (29 Refs)

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HE 20.3173/2:

2. DETECTION OF A TRANSFORMING GENE PRODUCT IN CELLS TRANSFORMED BY MOLONEY MURINE SARCOMA VIRUS.

Papkoff J, Verma IM, Hunter T
Dept. Biology, University California, San Diego, La Jolla, CA, 92093
Cell; 29(2):417-426 1982

We identified, in cells transformed by Moloney murine sarcoma virus (M-MuSV clone 124), a protein encoded by the M-MuSV transforming gene, v-mos. An antiserum against a synthetic peptide corresponding to the C terminus of a protein predicted from the v-mos nucleotide sequence specifically recognizes a protein doublet of approximately 37,000 daltons from 35S-methionine-labeled M-MuSV 124-transformed producer cells. By peptide mapping, this protein is almost identical to the 37 kd in vitro translation product from the M-MuSV v-mos gene. Immunoprecipitates from 32P-labeled cells contain a single v-mos-specific phosphoprotein, which has at least six sites of phosphorylation containing phosphoserine. Pulse-chase experiments show that the lower band in the 35S-methionine-labeled doublet is the primary translation product, which is modified, probably by phosphorylation, to yield the upper band. A similar mos protein is immunoprecipitated from HT1-MuSV-transformed cells, but not from uninfected NIH/3T3 cells. These mos proteins are present at very low levels in transformed cell lines. Cells acutely infected with M-MuSV 124, however, transiently contain much higher levels of the mos protein. These high levels coincide with extensive cell mortality. (Author abstract) (55 Refs)

3. PROTEINS SPECIFIED BY AVIAN ERYTHROBLASTOSIS VIRUS: CODING REGION LOCALIZATION AND IDENTIFICATION OF A PREVIOUSLY UNDETECTED ERB-B POLYPEPTIDE.

Privalsky ML, Bishop JM
Dept. Microbiology and Immunology, Univ. California, San Francisco, CA, 94143
Proc Natl Acad Sci USA; 79(13):3958-3962 1982

Avian erythroblastosis virus (AEV) induces erythroblastosis and sarcomas in chickens. Two domains within the viral genome, erb-A and erb-B, have been implicated in AEV-mediated oncogenesis. By use of hybridization-arrested translations and hybridization-selections of mRNA, we have mapped on the viral genome the polypeptides specified by the erb domains. The results of hybridization-arrest with DNA representing the spliced 5' leader region of the AEV mRNA suggested that the authentic product of the erb-B domain was a 61,000 mol wt protein, not a 41,000 mol wt polypeptide previously identified. (Author abstract) (28 Refs)

4. NUCLEAR LOCATION OF THE PUTATIVE TRANSFORMING PROTEIN OF AVIAN MYELOCYTOMATOSIS VIRUS.

Abrams HD, Rohrschneider LR, Eisenman RN
Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
Cell; 29(2):427-439 1982

The putative transforming protein of avian myelocytomatosis virus MC29 is a 110,000 dalton (P110gag-myc) polyprotein comprised of sequences derived from both the gag region and the MC29-specific myc region. Two approaches have been taken to determine the location of the MC29 gag-related proteins in transformed cells: subcellular fractionation and immunofluorescence. Analysis of subcellular fractions of MC29-transformed cells by immunoprecipitation indicates that the majority of the gag-myc polyprotein is found in the nuclear fractions of Q8 cells (a nonproducer line of MC29-transformed quail embryo fibroblasts) and nonproducer cells derived from a liver tumor of MC29-infected quail. This is in contrast to the distribution of gag-related helper virus proteins lacking myc, which are found only in nonnuclear fractions of superinfected Q8 cells. The purity of unlabeled nuclei was assessed by electron microscopy and enzyme assays, revealing little contaminating material from other subcellular fractions. Immunofluorescence experiments using monospecific anti-gag serum showed specific, intense immunofluorescence in the nuclei of fixed Q8 cells. In contrast, the majority of P75gag-erb, a candidate transforming protein produced by avian erythroblastosis virus (AEV), is absent from the nuclei of nonproducer AEV-transformed chick embryo fibroblasts. The nuclear association of the MC29 transforming protein may be related to some of the unique properties of MC29-transformed cells. (Author abstract) (68 Refs)

5. ASSOCIATION OF THE TRANSFORMING PROTEINS OF ROUS, FUJINAMI, AND Y73 AVIAN SARCOMA VIRUSES WITH THE SAME TWO CELLULAR PROTEINS.

Lipsich LA, Cutt JR, Brugge JS
Dept. Microbiology, State Univ. New York at Stony Brook, Stony Brook, NY, 11794
Mol Cell Biol; 2(7):875-880 1982

Association of proteins of mol wt 90,000 (pp990) and of 50,000 (pp50) with transforming proteins of Fuijinami sarcoma virus (FSV) and Yamaguchi 73 sarcoma virus (Y73) is demonstrated. To determine whether the pp90 and pp50 coprecipitated with the transforming proteins encoded by FSV and Y73, [35S]methionine-labeled cell lysates were first analyzed by direct immunoprecipitation. Both serum from rabbits bearing tumors induced by Rous sarcoma virus and anti-pp19gag sera precipitated structural proteins from FSV and Y73, transformation-defective Rous sarcoma virus (RSV), and nondefective RSV lysates. Three transformation-specific proteins were precipitated by the TBR serum from RSV-transformed cells. The two proteins, pp90 and pp50, coprecipitated with pp60src as a consequence of a physical association with pp60src. Immunoprecipitates of Y73-and FSV-transformed cell lysates incubated with antiserum to pp19gag were also found to contain proteins which comigrated with the pp9C and pp50 proteins, which associate with pp60src in RSV-transformed cell lysates. The pp90 and pp50 proteins were not precipitated from lysates of cells infected with transformation-defective virus, which are detected in the src gene, nor from uninfected chicken cells. Results strongly suggested that the cellular proteins pp90 and pp50 may have a common role in the mechanism of transformation induced by the sarcoma-inducing viruses which encode tyrosine-specific kinases. (17 Refs)

6. PROPERTIES OF THE SRC KINASE PURIFIED FROM ROUS SARCOMA VIRUS-INDUCED RAT TUMORS.

Richert ND, Blithe DL, Pastan I
Lab. Molecular Biology, NCI, NIH, Bethesda, MD, 20205
J Biol Chem; 257(12):7143-7150 1982

We recently described a method for the purification of a protein related to pp60src from Rous sarcoma virus-induced rat tumors. In this report, we describe some of the properties of the 7,200-fold purified enzyme. The purified kinase phosphorylates casein, vinculin, actin, and histone H2B, but not bovine serum albumin or ovalbumin. Protein substrates are phosphorylated exclusively on tyrosine residues. Casein was used as a substrate for more detailed analysis. The phosphorylation reaction proceeds at a linear rate for at least 40 min at 22

C. Maximum enzyme activity occurs at pH 6.5 to pH 6.8 and requires the presence of either Mg++ (5 to 10 mM) or Mn++ (1 to 5 mM). The Km for ATP is 30 uM and the Vmax is 0.03 umol/min/mg using 0.4 mg/ml of casein as a substrate. The enzyme utilizes ATP or dATP, but not GTP as a phosphate donor in the reaction. The enzyme is inhibited by adenosine and deoxyadenosine and by their corresponding mono- and diphosphates. No inhibition of enzyme activity is observed with adenine, GTP, UTP, TTP, or CTP. The enzyme is very sensitive to increased ionic strength. Addition of 0.1 M KCl, 0.1 M NaCl, 50 mM KPO4, or 50 mM NaPO4 inhibited casein phosphorylation by 90 to 95%. Analysis of the products of the phosphorylation reaction by thin layer chromatography revealed that the src kinase phosphorylates glycerol in addition to casein or the enzyme itself. (Author abstract) (27 Refs)

7. PURIFICATION OF A TYROSINE-SPECIFIC PROTEIN KINASE FROM ROUS SARCOMA VIRUS-INDUCED RAT TUMOR.

Blithe DL, Richert ND, Pastan IH
Lab. Molecular Biology, NIH, NCI, Bethesda, MD, 20205
J Biol Chem; 257(12):7135-7142 1982

We have identified a tyrosine kinase activity present in tumors which were raised in rats by subcutaneous injection of Rous sarcoma virus-transformed rat cells (SR-NRK). This kinase phosphorylates tyrosine on the heavy chain of IgG from tumor-bearing rabbit (TBR) sera specific for the src gene product, pp60src. Using TBR-IgG phosphorylation as an assay, we have purified this kinase over 7,200-fold. The purification procedure involves detergent extraction of tumors followed by sequential column chromatography on hydroxylapatite, DEAE-Sephacel, oligodeoxyadenosine-cellulose, an affinity column prepared from TBR-sera, and Sephacryl S-200. The IgG kinase activity behaves as a molecule of apparent Mr = 54,000 on Sephacryl S-200 molecular sieve chromatography. Analysis of the Sephacryl fractions by SDS-PAGE indicates that a major Coomassie blue-stained band with an apparent Mr = 54,000 (p54), co-elutes with the peak of kinase activity. From 600 g of tumors, approx 200 ug of p54 are obtained. We have four types of evidence which show that p54 is related to pp60src. (1) Purified p54 is capable of undergoing endogenous phosphorylation in the presence of [gamma-32P]ATP producing a 32P-labeled pp54 polypeptide which is specifically immunoprecipitated by TBR-sera and contains only phosphotyrosine. (2) Purified p54 competes with 32P labeled pp60src for binding to TBR-IgG, indicating a degree of purification over starting material which agrees very well with the results obtained by the IgG kinase assay. (3) V8 protease digestion of pp60src and p54 suggests that they share a common 26,000 fragment. (4) Antibodies to partially purified p54 specifically precipitate pp60src from Rous sarcoma virus-transformed chicken cells. (Author abstract) (45 Refs)

8. IN VIVO TYROSINE PHOSPHORYLATIONS OF THE ABELSON VIRUS TRANSFORMING PROTEIN ARE ABSENT IN ITS NORMAL CELLULAR HOMOLOG.

Ponticelli AS, Whitlock CA, Rosenberg N, Witte ON
Dept. Microbiology, Molecular Biology Inst., Univ. California, Los Angeles, CA, 90024
Cell; 29(3):953-960 1982

The transforming gene product of the Abelson murine leukemia virus (A-MuLV) is a phosphoprotein encoded by combined viral and cellular sequences. Previous work has shown the existence of a serologically crossreactive normal cellular phosphoprotein called NCP150. We have utilized two-dimensional phosphopeptide mapping and phosphoamino acid analysis to compare the structures of NCP150 and wild-type and mutant forms of the A-MuLV protein labeled in vivo with 32P-orthophosphate. This analysis demonstrated cellular homology between NCP150 and wild-type A-MuLV protein, but a number of phosphorylation differences were seen. Among them, two specific tyrosine phosphorylations present in all transformation-competent Abelson proteins were not observed in NCP150. No other phosphotyrosine-containing peptides were detected. In addition, transformation-defective mutants isolated from either the P120 or P160 wild-type strain lack phosphotyrosine-containing peptides. Double-infection studies with such transformation-defective and transformation-competent A-MuLV strains show that Abelson viral proteins may be substrates for their own tyrosine-specific kinase activity in vivo. These observations suggest that the phosphotyrosine kinase activity of the abl region may be controlled, and may function, differently in its viral and cellular forms. (Author abstract) (33 Refs)

VIRAL RNA

9. VIRUS-SPECIFIC TRANSCRIPTION AND TRANSLATION IN ORGANS OF BALB/MO MICE: COMPARATIVE STUDY USING QUANTITATIVE HYBRIDIZATION, IN SITU HYBRIDIZATION, AND IMMUNOCYTOCHEMISTRY.

Simon I, Lohler J, Jaenisch R
(c/o Jaenisch), Universitat Hamburg, Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie, Martinistrasse 52, 2000 Hamburg 20, W. Germany
Virology; 120(1):106-121 1982

Expression of the endogenous Moloney leukemia virus (=M-MuLV) was studied in a variety of tissues of BALB/Mo mice. Previous experiments using quantitative hybridization techniques have shown that M-MuLV expression occurred predominantly in the lymphatic organs spleen and thymus. Here we have employed in situ hybridization and immunocytochemical techniques to detect virus RNA and protein expression in single cells of a large variety of different organs. The two techniques were employed in parallel and compared with quantitative hybridization experiments using total nucleic acids extracted from the different organs. This allowed estimation of the fraction of positive cells in a given organ and to relate it to the total viral-specific RNA present, and thus to assess the value and the limitations of each method for detection of virus expression. The results confirm that most or all cells of the lymphatic system express regularly high concentrations of viral RNA and proteins. We show furthermore that cells of many nonlymphatic organs can be productively infected with virus expressing viral functions. These include cells of the reproductive system, epithelial cells of intestine, brain, and liver, and hormone-producing cells of the pancreas. With the exception of the reproductive system the fraction of virus-producing cells in all nonlymphatic organs, however, was much lower than in spleen and thymus. Our results indicated that productive infection with M-MuLV is not restricted to cells of specific tissues, although the frequency of infection may vary considerably. They furthermore demonstrate that regardless of cell type viral proteins are always synthesized once viral RNA has been transcribed. Our results suggest that active cell proliferation and hormone production may be among the cellular parameters

that influence susceptibility to productive virus infection. (Author abstract) (31 Refs)

10. THE VIRION RNA SPECIES OF THE KIRSTEN MURINE SARCOMA-LEUKEMIA VIRUS COMPLEX RELEASED FROM A CLONALLY RELATED SERIES OF MOUSE CELLS.

Clewley JP, Avery RJ
Dept. Biological Sciences, Univ. Warwick, Coventry, CV4 7AL, England
Arch Virol; 72(1/2):35-46 1982

We have characterized the virion RNA species of Kirsten sarcoma (KiSV) and Kirsten leukemia (KiLV) viruses released from a clonally related series of mouse cells (14). We have identified the KiLV and KiSV genome RNAs. In addition to the viral RNA species we find large amounts of a virus-like RNA (VL30 RNA), which is heterogeneous and shows variability in its expression. The amount of VL30 RNA in virions does not correlate with the state of transformation of the cells releasing the virus or the ability of the virus to transform other cells. Characterization of RNA rescued from non-producer cells has revealed a sarcoma virus (KiSVCB3) with an oligonucleotide fingerprint different from that of a standard KiSV RNA, suggesting that it has lost some viral sequences. The oligonucleotide fingerprints of KiLV and VL30 RNAs are distinct from each other and from those reported for other murine leukemia virus RNAs. (Author abstract) (21 Refs)

REVERSE TRANSCRIPTION, VIRAL DNA INTERMEDIATES, AND PROVIRAL DNA

11. DE NOVO METHYLATION, EXPRESSION, AND INFECTIVITY OF RETROVIRAL GENOMES INTRODUCED INTO EMBRYONAL CARCINOMA CELLS.

Stewart CL, Stuhlmann H, Jahner D, Jaenisch R
Heinrich-Pette-Instut fur Experimentelle Virologie und Immunologie, Universitat Hamburg, Martinistrasse 52, 2000 Hamburg 20, W. Germany
Proc Natl Acad Sci USA; 79(13):4098-4102 1982

We have investigated the block to expression of Moloney murine leukemia virus in murine embryonal carcinoma (EC) cells. Infected EC cells were found to contain up to 100 integrated proviral genomes. However, expression of virus as measured by XC plaque and virus-specific RNA synthesis did not occur at significant levels, in contrast to productively infected differentiated cells. Analysis of the DNA in the infected EC cells revealed that the proviral genomes were highly methylated, as shown by their resistance to cleavage by SmaI. Integrated proviral genomes in infected differentiated cells were readily cut by Sma I and thus were not methylated at these sites. Transfection of DNA from infected EC cells to cells permissive for virus expression failed to induce virus expression. The proviral genomes, however, were potentially infectious because they induced XC plaques when the recipient cells for transfection were treated with 5-azacytidine. This drug is believed to interfere with DNA methylation. We conclude that expression of proviral genomes introduced into EC cells is suppressed and that this inactivation can be correlated with the de novo methylation of the viral DNA. De novo methylation activity thus may be a characteristic of early embryonic cells. (Author abstract) (43 Refs)

12. THE CHANGES IN PROVIRAL CHROMATIN THAT ACCOMPANY MORPHOLOGICAL VARIATION IN AVIAN SARCOMA VIRUS-INFECTED RAT CELLS.

Chiswell DJ, Gillespie DA, Wyke JA
Amersham International, Amersham, Bucks, England
Nucleic Acids Res; 10(13):3967-3980 1982

The clone All of avian sarcoma virus B77-infected Rat-1 cells comprises both morphologically normal and morphologically transformed derivatives. Transformed subclones, in which virus-specific RNA is readily detectable, contain a provirus that is very sensitive to DNase I digestion of chromatin, and show DNase I hypersensitive sites at the 5' end of the provirus and in 5' flanking cell DNA. Normal subclones with no detectable virus-specific RNA, whether infected cells that have never been transformed or revertants derived from transformed cells, contain a provirus that is far more resistant to DNase I digestion. Moreover this provirus lacks hypersensitive sites at its 5' end, although DNase I hypersensitive sites are present in 5' flanking cell DNA. No DNase I hypersensitive sites were detected at the 3' end of the provirus in either normal or transformed clones. The pattern of cytosine methylation in the proviral restriction sites of the isoschizomers Msp I and Hpa II differed between transformed and revertant clones; the revertants show additional methylation at some CpG doublets. (Author abstract) (42 Refs)

13. DETECTION OF ACQUIRED PROVIRUS SEQUENCES IN MAMMARY TUMORS FROM LOW-EXPRESSOR, LOW-RISK MICE.

Altrock BW, Cardiff RD, Puma JP, Lund JK
(c/o Cardiff) Dept. Pathology, Sch. Medicine, Univ. California, Davis, Davis, CA, 95616
JNCI; 68(6):1037-1041 1982

Murine mammary tumor virus (MuMTV) provirus sequences in the DNA from early-occurring (av age 10 mo) and late occurring (age greater than 20 mo) tumors in BALB/cfC3H mice were analyzed by Eco RI restriction endonuclease mapping procedures. All early tumors were MuMTV antigen-positive mammary adenocarcinomas that contained the 0.92-and 4.0-kilobase (kb) exogenous C3H MuMTV-specific Pst I restriction endonuclease fragments. All but one of the late mammary adenocarcinomas had MuMTV antigens detected by peroxidase antiperoxidase staining, and all contained the 0.92-and 4.0-kb exogenous virus Pst I fragments. Three late nonmammary tumors lacked both MuMTV antigens and acquired provirus sequences. Greater numbers of MuMTV sequences were detected in both early and late-arising mammary tumors by Eco RI restriction endonuclease mapping than were detected in tissues from uninfected BALB/c mice. However, neither the number nor the location of MuMTV proviruses correlated with tumor latent period. (Author abstract) (19 Refs)

14. COMMON PROVIRAL INTEGRATION SITES IN C57BL MOUSE LYMPHOMAS INDUCED BY RADIATION LEUKEMIA VIRUS AND ABSENCE OF NOVEL VIRUS-RELATED SEQUENCES IN RADIOGENIC LYMPHOMA DNA.

Janowski M, Boniver J, Maisin JR
Dept. Radiobiology, Centre d'Etude de l'Energie Nucleaire, B-2400 Mol, Belgium
Leuk Res; 6(3):285-297 1982

Provirus topography was investigated in radiation leukemia virus (RadLV)- and RadLV/VL3-induced C57BL/Ka lym-

phoma cell lines, in radiogenic C57/BL and C57/BL/6 lymphomas, and in radiogenic C57BL/Ka lymphoma cell lines. The EcoRI and BamHI restriction patterns of BL/VL3 cells DNAs were investigated using radioactive probes synthesized from RadLV/VL3 and from BL/F(SL) RNAs. The results obtained with either probe were indistinguishable from each other. BL/VL3 clones displayed several virus-related EcoRI-generated fragments absent in control C57BL/Ka DNA indicating that the BL/VL3 cell line, and hence probably the tumor from which it was derived, is the result of the proliferation of cells where provirus integration occurred at characteristic DNA sites. Six EcoRI restriction patterns of thymus and bone marrow cell cultures from RadLV/VL3 induced tumor were identical showing novel virus-related fragments all longer than 10 kilobase pairs (kb). The six BamHI patterns were also indistinguishable, with novel virus-specific fragments of approx 3, 5, 6, and 12.5 kb. As in the case of BL/VL3, the restriction patterns of the thymus and bone marrow tumor cells suggested the presence of more than one provirus. Thymic lymphomas were induced by a scheduled irradiation scheme in two different C57BL mouse strains. Sixteen individual C57BL/6 and three individual C57/BL tumors were investigated by DNA restriction enzyme analysis. In addition, cloned and uncloned cell lines, either virus producers or not, derived from C57/BL/Ka radiogenic tumor were examined. In neither case did EcoRI analysis reveal the presence of virus-related sequences absent from control DNA. (33 Refs)

15. NOVEL CLASS OF MOUSE MAMMARY TUMOR VIRUS-RELATED DNA SEQUENCES FOUND IN ALL SPECIES OF MUS, INCLUDING MICE LACKING THE VIRUS PROVIRAL GENOME.

Callahan R, Drohan W, Gallahan D, D'Hoostelaere L, Potter M
Lab. Cellular and Molecular Biology, NCI, NIH, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 79(13):4113-4117 1982

Mice in breeding colonies of feral Mus musculus brevirostris (Azrou, Morocco), M m musculus (Studenec, Czechoslovakia), and M m molossinus (Fukuoka, Japan) were found to lack the mouse mammary tumor virus (MMTV-alpha) proviral genome in their germ line. MMTV-alpha proviral genomes have been found in all inbred strains of M musculus by using high-stringency nucleic acid hybridization conditions. We conclude that feral mice in these colonies are heterozygous for a limited number of MMTV-alpha proviral genomes and that those lacking them arose as a result of random chromosomal segregation. All mice in another breeding colony of feral M m musculus (Sladeckovce, Czechoslovakia) lack MMTV proviral genes. By relaxing the conditions of nucleic acid hybridization, MMTV-related sequences (designted MMTV-beta) were detected in restricted cellular DNA from MMTV-negative mice and all other inbred strains and feral species of the genus Mus. The apparent ubiquity of the MMTV-beta DNA sequences in the genus Mus and the lack of variation in the pattern of restriction fragments containing these sequences within a species distinguishes from from MMTV-alpha. These results suggest that the MMTV-beta DNA sequences either are the evolutionary progenitors of the infectious MMTV genome or represent an accumulation of evolutionarily divergent MMTV-alpha insertions into the mouse germ line. (Author abstract) (35 Refs)

16. T24 HUMAN BLADDER CARCINOMA ONCOGENE IS AN ACTIVATED FORM OF THE NORMAL HUMAN HOMOLOGUE OF BALB- AND HARVEY-MSV TRANSFORMING GENES.

Santos E, Tronick SR, Aaronson SA, Pulciani S, Barbacid M
Lab. Cellular and Molecular Biology, NCI, NIH, Bethesda, MD, 20205
Nature; 298(5872):343-347 1982

A transforming gene isolated from T24 human bladder carcinoma cells is closely related to the BALB murine sarcoma virus (MSV) onc gene (v-bas). This transforming gene is localized to a 4.6 kilobase pair (kbp) region and is expressed as a 1.2-kbp polyadenylated transcript, which contains v-bas related sequences. Moreover, antisera known to detect the immunologically related onc gene products of BALB- and Harvey-MSV recognized elevated levels of a related protein in T24 cells. The normal human homologue of v-bas was found to be indistinguishable from the T24 oncogene by heteroduplex and restriction enzyme analysis. These results imply that rather subtle genetic alterations have led to the activation of the normal human homologue of v-bas as a human transforming gene. (Author abstract) (48 Refs)

OTHER RELATED STUDIES

17. AVIAN LEUKEMIA VIRUSES ONCOGENES AND GENOME STRUCTURE.

Graf T, Stehelin D
Inst. Virology, German Cancer Res. Center, In Neuenheimer Feld 280, 6900 Heidelberg, W. Germany
Biochim Biophys Acta; 651(4):245-271 1982

Avian leukemia viruses, with emphasis mainly on defective leukemia viruses (DLVs), are reviewed. Oncogenic spectra of chicken-derived DLVs and the turkey-derived reticuloendotheliosis virus (REVT) are presented. Transformation specificity of DLVs in vitro, genetic content and genome structure of avian erythroblastosis virus (AEV), avian myeloblastosis virus (AMV)-type, myelocytomatosis virus strain 29 (MC29)-type, REVT, expression of RNA and proteins in cells infected with AEV, AMV, MC29, and OK10, genetics of DLVs including mutants in transforming functions and recovered viruses, conservation of cellular oncogene (c-onc) genes through evolution, chromosomal localization and structure of c-onc genes, and expression of c-onc genes in normal cells, mechanisms of transformation by avian leukosis viruses (ALV) and DLVs, the promoter insertion hypothesis of ALV-leukemogenesis, hematopoietic target cell specificity and possible role of c-onc genes in the DLV-leukemogenesis, and transformation of nonhematopoietic cells by DLVs are discussed. Origin of DLVs is reviewed, including reverse transcription of encapsidated viral and cellular RNAs, viral promoter insertion, recombination of integrated provirus with a c-onc gene, and integration of proviruses around a c-onc gene. (167 Refs)

18. ENDOGENOUS RETROVIRUSES OF MICE AND CHICKENS.

Steffen DL, Robinson H
Worcester Foundation Experimental Biology, 222 Maple Ave., Shrewsbury, MA, 01545
Curr Top Microbiol Immunol; 98:1-10 1982

Characteristics of murine leukemia viruses (MLV) found in laboratory mice and avian leukosis viruses (ALV) found in chickens are reviewed. These viruses occur as endogenous retroviruses (ERV) in the host animals. ALV proviruses constitute one closely related family. MLV constitute two or more families. ALV occur as 0-5 copies in the DNA of chickens, while MLV are represented by a larger number of copies. ALV are relatively easy to analyze structurally; analysis of MLV proviruses is more complicated, and therefore fewer MLV have been analyzed. Five structural classes of ALV have been identified; three of these classes have been identified among MLV. ERV occur throughout the host genome, indicating that infection is the source of ERV. The number of copies of endogenous proviruses in the host genome may increase over time; this increase probably results from reinfection. Endogenous proviruses may genetically complement or recombine with exogenous or endogenous infecting viruses. Expression of endogenous proviruses is apparently controlled by methylation of DNA. Chicken and mouse hosts display resistance to ERV. Resistance at the level of adsorption and penetration evidently results from loss of surface receptors by the host cell. Resistance may also develop at a level after penetration. Current evidence suggests that the most evolved proviruses are the most widely distributed; xenotropic MLV are the most highly selected, while ALV are at an intermediate stage of selection, and endogenous ecotropic MLV are the least evolved. (25 Refs)

19. THE GENETIC BASIS OF RETROVIRAL-INDUCED TRANSFORMATION.

Robinson HL, Vande Woude GF
Worcester Foundation Experimental Biology, 222 Maple Ave., Shrewsbury, MA, 01545
Curr Top Microbiol Immunol; 98:11-16 1982

The interaction of retroviruses with oncogenic sequences was studied in vitro and in vivo. In vitro studies with the Moloney sarcoma virus (MSV) indicated that the long terminal repeats (LTR) of MSV have transcriptional control sequences and structures that resemble insertion sequences. LTR enhanced transformation by an MSV DNA fragment containing the v-mos sequence (a viral homolog of the cellular c-mos sequence). Enhancement of transformation was accomplished either by directly linking the LTR and v-mos or by simultaneously transfecting a plasmid containing a single LTR with a plasmid containing v-mos. LTR may therefore enhance transformation by providing transcriptional control signals. LTR also activates transformation by c-mos. Transformation of cells by MSV apparently requires c-mos and LTR. Oncogenesis was studied in vivo by infecting K28 chickens with RAV-0 endogenous virus or with NTRE-7 (a nontransforming subgroup E recombinant of exogenous Pr-RSV-B and RAV-0 viruses). Of 58 chickens infected with RAV-0, none developed disease. Of 75 chickens infected with NTRE-7, 11 developed neoplasias (3 lymphomas, 2 fibrosarcomas, 2 carcinomas, 1 chondrosarcoma, and 3 osteopetroses). RAV-0 and NTRE-7 differ only in the immediate 3' sequences; therefore these sequences contain transcriptional controls for synthesis of viral messenger RNA that determine oncogenic potential in NTRE-7. Findings support a model of viral oncogenesis in which oncogenesis is controlled by the region of the viral genome that encodes control of transcription, and the type of neoplasia produced is determined by specific 3' oncogenic sequences. (16 Refs)

20. EXPRESSION OF XENOTROPIC MURINE LEUKEMIA VIRUSES.

Morse HC, Hartley JW
Lab. Microbial Immunity, Natl. Inst. Allergy and Infectious Diseases, Bethesda, MD, 20014
Curr Top Microbiol Immunol; 98:17-26 1982

Expression of xenotropic murine leukemia viruses (X-MuLV) and X-MuLV-encoded glycoprotein 70 (gp70) antigens was studied in various strains of mice. Genetic information from X-MuLV was found in all strains of mice; expression of this information as infectious virus or gp70 antigens was negatively controlled in most strains. In strains that express high levels of infectious MuLV, negative control was overcome in a variety of ways. Expression of infectious MuLV was not related to the strain of mouse. Expression of MuLV as infectious virus or X-MuLV-coded cell surface antigens (XenCSA) was determined by different factors. In most inbred strains of mice levels of XenCSA were controlled by genes that govern differentiation of T-lymphocytes. Expression of X-MuLV information in T-cells was not related to expression in B-cells. Crosses between inbred strains show different forms of genetic control of the expression of X-MuLV. Findings show no evidence that the products of X-MuLV genes play a role in any normal physiological process. Such a role is, however, not excluded by the evidence. (29 Refs)

21. EXPRESSION OF ENDOGENOUS VIRAL GENES IN MOUSE LYMPHOCYTES.

Wecker E, Horak I
Institut fur Virologie und Immunbiologie, Universitat Wurzburg, Versbacher Strasse 7, D-8700 Wurzburg, W. Germany
Curr Top Microbiol Immunol; 98:27-36 1982

The association of expression of endogenous viral genes of xenotropic origin with immunological phenomena in mice and chickens is discussed. Xenotropic murine leukemia virus (X-MuLV) genes are present in all strains of mice. Viral gene products, such as X-MuLV gp70, may be expressed spontaneously or after stimulation by mitogens or antigens. In some strains of mice, stimulation of B-lymphocytes by mitogens leads to differentiation to immunoglobulin-producing cells, accompanied by production of C-type virus particles. T-cells can also be stimulated to express MuLV gp70 and env gene products. X-MuLV gp70 is expressed to widely varying degrees by lymphocytes from greater than 100 strains of mice studied. Stimulation by B- or T-cell mitogens enhances this expression. In most strains of mice expression of env genes is predominantly repressed. Activation of lymphocytes results in derepression of env genes. Some evidence suggests that gp70 or other X-MuLV gene products may play some role in normal physiological processes in mice and chickens. Several studies indicate that both mouse and chicken proviral sequences have long 5' and 3' terminal repeats (LTR). These LTR are capable of promoting transformation. If X-MuLV viruses have similar promoting activity, LTR may be more important than structural viral genes in promoting growth and/or differentiation of host cells, particularly lymphocytes. (24 Refs)

22. ONCOVIRAL PROTEINS AS CELLULAR ANTIGENS.

Fleissner E, Snyder HW
Memorial Sloan-Kettering Cancer Inst., 1275 York Ave., New York, NY, 10021
Curr Top Microbiol Immunol; 98:37-80 1982

The immunological recognition of proteins encoded by oncoviruses and the biological consequences of recognition are reviewed. Virally produced proteins include structural proteins of the virion, variants of these proteins that do not reside on the virions, and proteins that are coded by cellular genes that have become incorporated into the oncoviral genome. The last-mentioned type of protein can produce malignant transformation. Many viral proteins are found on the cell surface membrane and therefore act as antigens. As antigens, the proteins provide a target for immune response by the host. The immune response may be useful for in vitro serological analysis. The murine system has provided a model for study of immune response by the host to virion structural proteins and the expression of endogenous viral proteins in various hematopoietic cell lineages. The feline system has been a model for assessment of host responses to virally-induced malignancy. The feline system has produced the best evidence that an immune response by the host to viral transformation antigens can be decisive in rejection of a tumor and indicative of the incidence of a tumor in a population. Discussion emphasizes recognition in the host species from which particular viruses were derived. Only C-type viruses are discussed. (400 Refs)

23. POSSIBLE IMMUNOLOGICAL MECHANISMS IN C-TYPE VIRAL LEUKEMOGENESIS IN MICE.

Ihle JN, Lee JC
Biological Carcinogenesis Program, Frederick Cancer Res. Center, Frederick, MD
Curr Top Microbiol Immunol; 98:85-101 1982

The role of C-type viruses in leukemogenesis in mice is reviewed. The mechanisms by which C-type viruses induce leukemia are distinct from those by which the same type of viruses induce sarcomas. In leukemogenesis the viruses are replication-competent, nontransforming, and do not contain additional cellular pathogenic sequences. Latency periods are longer for leukemia than for sarcomas. Induction of leukemia is associated with acute viremia. Studies in mice indicate cellular immune response is an integral part of the process of leukemogenesis. Among the specific components of the leukemogenic process is the absolute requirement for antigen-specific lymphocytes. Preleukemic states involve chronic immune stimulation that is not limited to antigen-specific components but includes systemic effects. A proposed model suggests that immune stimulation is required to provide targets for viral infection and transformation. A second model describes a unique interaction of virus with antigen-specific cells to produce transformation. In a third proposed model, chronic immune stimulation produces somatic errors, which result in transformation leading to leukemia. Experimental approaches are available for future research to clarify the mechanisms of viral leukemogenesis. (63 Refs)

24. PERSPECTIVES ON ENDOGENOUS RETROVIRUSES IN NORMAL AND PATHOLOGICAL GROWTH.

Weiss RA
Inst. Cancer Res., Fulham Road, London SW3 6JB, England
Curr Top Microbiol Immunol; 98:127-132 1982

The origin and persistence of endogenous retroviruses, normal functions of endogenous viruses, and viral leukemogenesis are reviewed. The endogenous viral elements studied in extant hosts are guilty of infection before their adoption by the germ line. The number, structure, and location of endogenous viral elements in individual hosts are variable due to substantial population polymorphism. Xenotropism is discussed. Endogenous proviruses are not significantly harmful to the host population. Env gene products may control intercellular signals involving interleukins and other lymphokines in certain species, but may not be universal. Intercellular communication may change from species to species. In some species endogenous retrovirus genes may have a useful lymphoid function. Except for certain high cancer strains of mice, endogenous retroviruses are not known to be pathogenic. An endogenous virus may evolve leukemogenic properties on exogenous passage, and the presence of endogenous sequences may protect the host from the oncogenic effect of a related exogenous virus. Low or non-leukemogenic endogenous viruses may be distinguished from the more potently oncogenic recombinant or exogenous relatives by the 3'-end of the genome, either in the env gene or the adjacent retrovirus flanking sequences. The latter region, rather than the env determines oncogenicity. The preneoplastic transformation of bursal follicles occurs faster and more often than the final, clonal development of metastatic lymphoma in chickens. Two hypotheses on leukemogenesis are described. (38 Refs)

25. BIOLOGICAL CARCINOGENESIS.

Rich MA, Furmanski F
AMC Cancer Res. Center and Hosp., Lakewood, CO
Biological Carcinogenesis. New York, Marcel Dekker, Inc., 310 pp., 1982.

The general principles which govern carcinogenesis are identified for each of the major classes of carcinogens and the extent to which these principles are applicable to the carcinogenic process induced by other classes of agents is determined. Current knowledge on the interaction of carcinogens and the identification of biological systems where viral, chemical, and physical carcinogens are demonstrably interactive are described. Mechanisms in viral carcinogenesis are discussed with respect to: T-cell growth, T-cell growth factor, T-cell leukemias and lymphomas and isolation of a new type-C retrovirus, properties of sarcoma growth factors produced by sarcoma virus transformed cells, and interactions of murine leukemia viruses with hematopoietic stem cells. Mechanisms in chemical carcinogenesis are discussed with respect to activation of carcinogenic polycyclic hydrocarbons by metabolism, involvement of a protein kinase in multistage skin tumor promotion, and the effect of tumor-causing chemicals on the control of mutagenesis and differentiation in mammalian cells. Transformation of cloned mammary epithelial cells following combined treatment with mouse mammary tumor virus and dimethylbenz(a)anthracene, virus-cell interactions during hormonal carcinogenesis, and separate pathways for viral and chemical carcinogenesis in the mouse mammary gland are described. A molecular approach to studies of chemical carcinogen-virus interaction is presented. Other presentations deal with multistage recombinational origin of leukemogenic viruses, radiation-induced leukemogenesis, neoplastic transformation of cultured cells by herpes simplex virus type 2 and carcinogens, modulation of adenovirus transformation and replication, and transformation of murine fibroblasts. (682 Refs)

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- ☐ The Role of Dietary Nitrate and Nitrite in Human Carcinogenesis (OK-82/01)
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